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Goeddel et al.

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(54) **MICROBIAL PRODUCTION OF MATURE HUMAN LEUKOCYTE INTERFERONS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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Related U.S. Application Data

(63) Continuation of application No. 06/703,148, filed on Feb. 19, 1985, now abandoned, which is a division of application No. 06/256,204, filed on Apr. 21, 1981, which is a continuation-in-part of application No. 06/205,578, filed on Nov. 10, 1980, now abandoned, which is a continuation-in-part of application No. 06/184,909, filed on Sep. 8, 1980, now abandoned, which is a continuation-in-part of application No. 06/164,986, filed on Jul. 1, 1980, now abandoned.

(51) **Int. Cl.**⁷ **C12P 21/02**

(52) **U.S. Cl.** **435/69.51**; 435/69.1; 435/252.3; 435/252.33; 536/23.52

(58) **Field of Search** 435/69.51, 172.3, 435/243, 252.3, 252.33, 320.1; 536/27, 23.52; 424/85.7

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(57) **ABSTRACT**

Disclosed herein are methods and means of microbially producing, via recombinant DNA technology, mature human leukocyte interferons, useful in the treatment of viral and neoplastic diseases.

22 Claims, 19 Drawing Sheets

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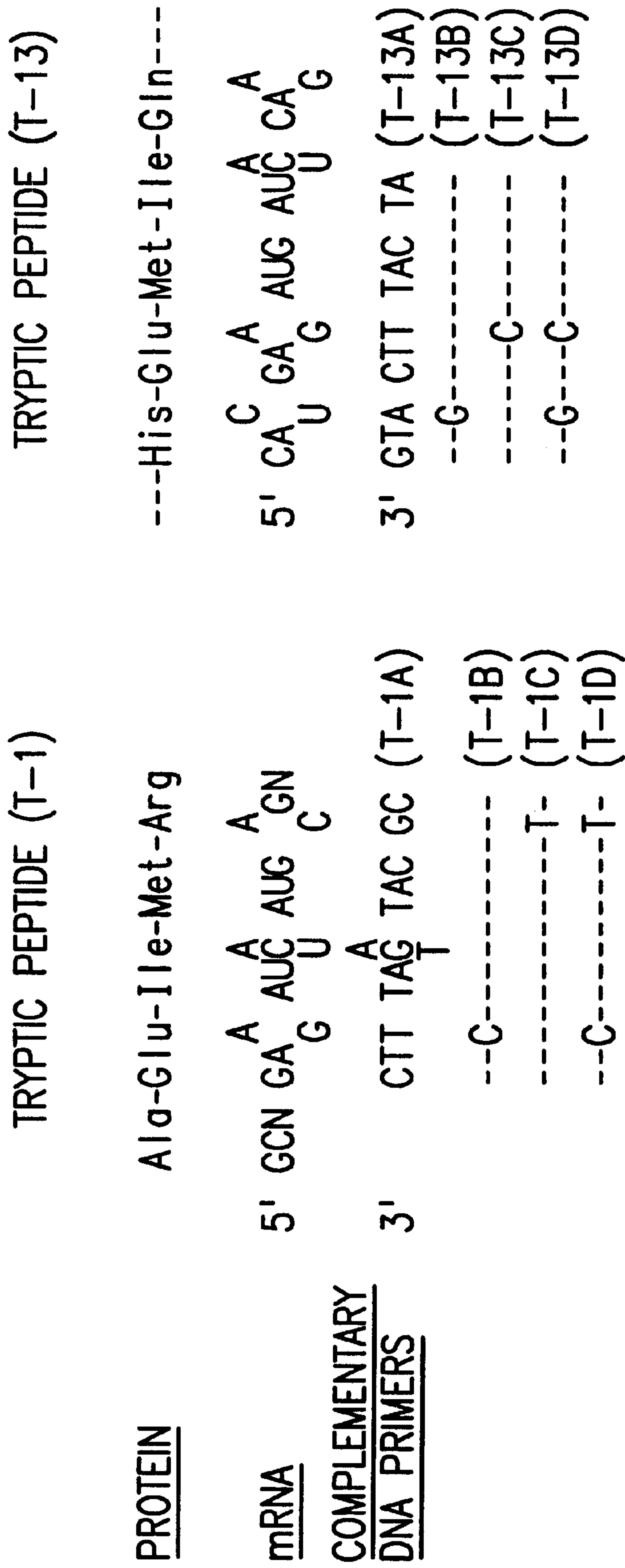


FIG.1

^{32}P -T-1C probe



^{32}P -T-13C probe

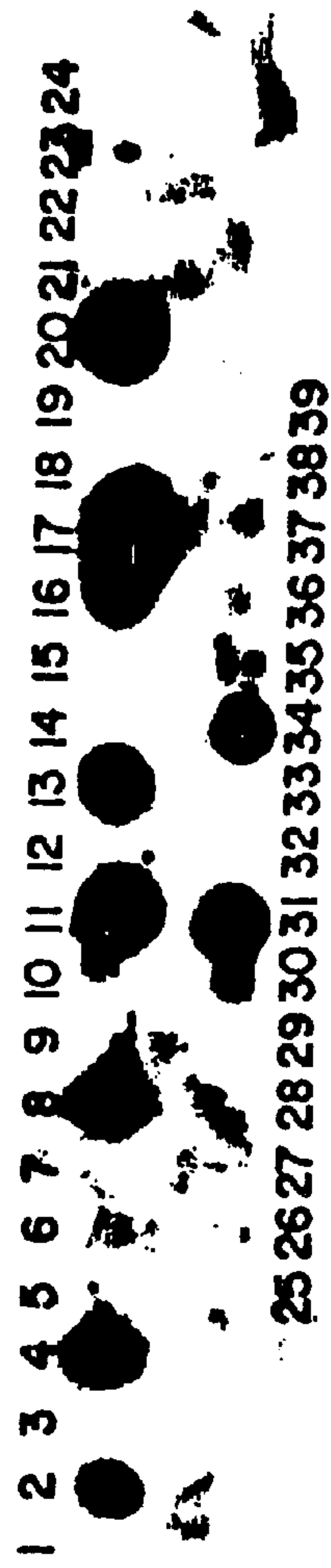


FIG. 2

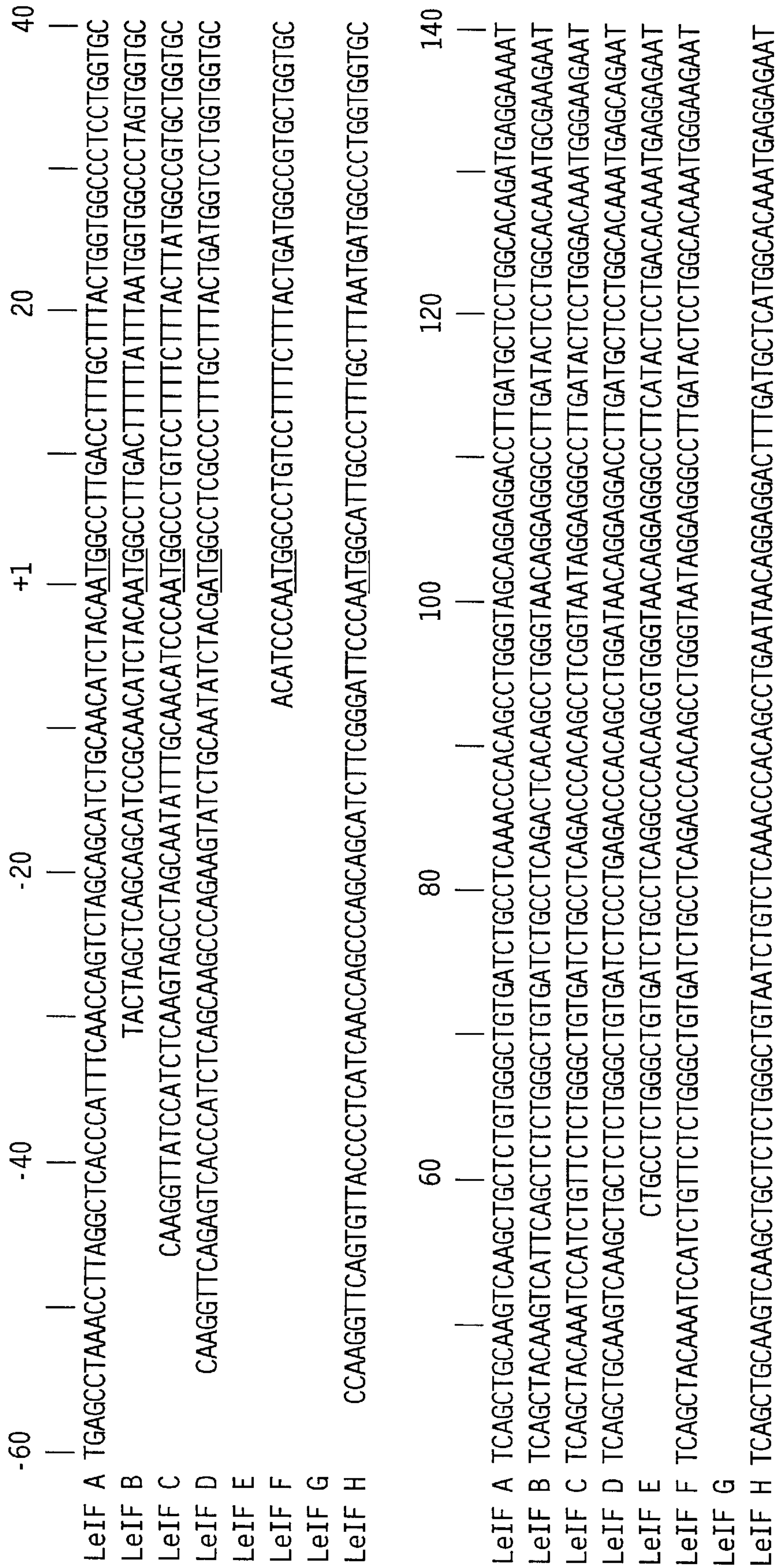


FIG. 3A

	160	180	200	220	240
LeIF A	CTCTCTTTTCTCCTGCTTGAAGGACAGACATGACTTTGGATTTCCC	CAGGAGGAGTTT	GGCAACCAGTTC	CAAAAGGCTGAAACCATCCCTGTCTCT	
LeIF B	CTCTCCTTTTCTCCTGCTGCTGAGGACAGACATGACTTTGAATTTCCC	CAGGAGGAGTTT	GATGATAAACAGTTC	CAGAAGGCTCAAGCCATCTCTGTCTCT	
LeIF C	CTCTCCTTTTCTCCTGCTGCTGAGGACAGACATGATTTCCGAATCCC	CAGGAGGAGTTT	GATGGCAACCAGTTC	CAGAAGGCTCAAGCCATCTCTGTCTCT	
LeIF D	CTCTCCTTTTCTCCTGCTGCTGATGGACAGACATGACTTTGGATTTCCC	CAGGAGGAGTTT	GATGGCAACCAGTTC	CAGAAGGCTCCAGCCATCTCTGTCTCT	
LeIF E	CTCTCCTTTTCTTACCTGAAGGACAGACATGACTTTGATTTTCCATCATCAGGTGTTT	CATGCAACCAC	TTCAGAAAGGTTCAAGCTATCTTCTCTTTT		
LeIF F	CTCTCCTTTTCTCCTGCTGCTGAGGACAGACATGACTTTGGATTTCCC	CAGGAGGAGTTT	GATGGCAACCAGTTC	CAGAAGGCTCAAGCCATCTCTGTCTCT	
LeIF G		CATGACTTTGGATTTCTCT	CAGGAGGAGTTT	GATGGCAACCAGTTC	CAGAAGGCTCAAGCCATCTCTGTCTCT
LeIF H	CTCTCCTTTTCTCCTGCTGCTGAGGACAGACATGACTTTGAATTTCCC	CAGGAGGAGTTT	GATGGCAACCAGTTC	CAGAAGGCTCAAGCCATCTCTGTCTCT	
	260	280	300	320	340
LeIF A	CCATGAGATGATCCAGCAGATCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTGCTTGGGATGAGACCCCTCCTAGACAAAATTTCTACACTGAACTCTAC				
LeIF B	CCATGAGATGATCCAGCAGACCTTCAACCTCTTCAGCACAAAGGACTCATCTGCTGCTTGGATGAGACCCCTTCTAGATGAAATTTCTACATCGAACTTGAC				
LeIF C	CCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAGAGGACTCATCTGCTGCTTGGGACACAGAGCCCTCCTAGAAAATTTTCCACTGAACTTTAC				
LeIF D	CCATGAGCTGATCCAGCAGATCTTCAACCTCTTTACCACAAAAGATTCATCTGCTGCTTGGGATGAGGACCTCCTAGACAAAATTTCTGCACCGAACTCTAC				
LeIF E	CCATGAGATGATGCAGCAGACCTTCAACCTCTTCAGCACAAAGGACTCATCTGATACCTTGGGATGAGACCCCTTTTAGACAAAATTTCTACACTGAACTTTAC				
LeIF F	CCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTACTTGGGACACAGAGCCCTCCTAGAAAATTTTCCACTGAACTTAAAC				
LeIF G	CCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTACTTGGGATGAGACACTTCTAGACAAAATTTCTACACTGAACTTTAC				
LeIF H	CCATGAGATGATGCAGCAGACCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTGCTTGGGATGAGACCCCTCCTAGAAAATTTCTACATTTGAACTTTTC				

FIG. 3B

360 380 400 420 440
 | | | | |
 LeIF A CAGCAGCTGAATGACCTGGAAGCCTGTGTGATACAGGGGGTGGGGGTGACAGAGACTCCCCTGATGAAGGAGGACTCCATTCTGGCTGTGAGGAAATACT
 LeIF B CAGCAGCTGAATGACCTGGAAGTCCCTGTGTGATCAGGAAGTGGGGGTGATAGAGTCTCCCCTGATGTACGAGGACTCCATCCCTGGCTGTGAGGAAATACT
 LeIF C CAGCAACTGAATGACCTGGAAGCATGTGTGATACAGGAGGTTGGCGTGGAAAGAGACTCCCCTGATGAATGAGGACTCCATCCCTGGCTGTGAGGAAATACT
 LeIF D CAGCAGCTGAATGACTTGGAAAGCCTGTGTGATGCAGGAGGAGGGTGGGAGAAACTCCCCTGATGAATGTGGACTCCATCTTGGCTGTGAAGAAATACT
 LeIF E CAGCAGCTGAATGACCTGGAAGCCTGTGTGATGTAGAGGTTGGAGTGGAAAGAGACTCCCCTGAGGAAATGTGGACTCCATCCCTGGCTGTGAGAAATACT
 LeIF F CAGCAGCTGAATGACATGGAAGCCTGCCTGATACAGGAGGTTGGGTGGAAAGAGACTCCCCTGATGAATGTGGACTCCATCTTGGCTGTGAAGAAATACT
 LeIF G CAGCAGCTGAATGACCTGGAAGCCTGTATGATGCAGGAGGTTGGAGTGGAAAGACACTCCTCTGATGAATGTGGACTCTATCCCTGACTGTGAGAAATACT
 LeIF H CAGCAAATGAATGACCTGGAAGCCTGTGTGATACAGGAGGTTGGGTGGAAAGAGACTCCCCTGATGAATGAGGACTCCATCCCTGGCTGTGAAGAAATACT

460 480 500 520 540
 | | | | |
 LeIF A TCCAAGAATCACTCTCTATCTGAAGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGTGAGAGCAGAAATCATGAGATCTTTTCTTTGTCAACAAA
 LeIF B TCCAAGAATCACTCTATATCTGACAGAGAAGAAATACAGCTCTTGTGCCTGGGAGGTTGTGAGAGCAGAAATCATGAGATCCTTCTCTTTATCAATCAA
 LeIF C TCCAAGAATCACTCTTTATCTAATAGAGAGGAAATACAGCCCTTGTGCCTGGGAGGTTGTGAGAGCAGAAATCATGAGATCCCTCTCGTTTTCAACAAA
 LeIF D TCCGAAGAATCACTCTCTATCTGACAGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGTGAGAGCAGAAATCATGAGATCCCTCTCTTTATCAACAAA
 LeIF E TTCAAAGAATCACTCTTTATCTGACAAAGAAGTAGTATAGCCCTTGTGCCTGGGAGGTTGTGAGAGCAGAAATCATGAGATCCTTCTCTTTATGAACGAA
 LeIF F TCCAAGAATCACTCTTTATCTGACAGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGTGAGAGCAGAAATCATGAGATCCTTCTCTTTATCAAAAAT
 LeIF G TTCAAAGAATCACCCCTCTATCTGACAGAGAAGAAATACAGCCCTTGTGCATGGGAGGTTGTGAGAGCAGAAATCATGAGATCCTTCTCTTTATCAGCAA
 LeIF H TCCAAGAATCACTCTTTATCTGATGGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGTGAGAGCAGAAATCATGAGATCCTTCTCTTTTCAACAAA

FIG. 3C

	560	580	600	620	640
LeIF A	CTTGCAAGAAAGTTTAAGAAAGTAAGGAAATGAAACTGGTTCAACATGGAAATGATTTTCATTGATTCGGATGCCAGCTCACCTTTTTATGATCTGCCATT				
LeIF B	CTTGCAAAAAGATTGAAGAGTAAGGAAATGAGACCTGGTACAACACGGAAATGATTTCTCATAGACTAATACAGCAGTCTACACTTTGACAAGTTGTGCTC				
LeIF C	CTTGCAAAAAGATTAAAGGAGGAAGGATTGAAAACCTGGTTCAACATGGCAATGATCCIGATTGACTAATACATTAATCTCACACTTTTCATGAGTTCTTCCA				
LeIF D	CTTGCAAGAAAGATTAAAGGAGGAAGGAAATAATCTGGTCCAAACATGAAACAATTTCTATTGACTCATAACACCAGGTCACGCTTTTCATGAATTTCTGTCA				
LeIF E	CTTGCAAGAAAGATTAAAGGAGGAAGGAAATGAAAACCTGGTTCAACATGGAAATGAGAAACATTTCCATGATTAATACATCATCTCACACATTCATGAATTC				
LeIF F	TTTTCAAGAAAGATTAAAGGAGGAAGGAAATGAAAACCTGGTTCAACATGGAAATGATCTGTATTGACTAATACACCAGTCCACACTTCTATGACTTCTGCCAT				
LeIF G	CTTGCAAGAAAGATTAAAGGAGGAAGGAAATGAAAACCTGGTTCAACATCGAAATGATTTCTCATTTGACTAGTACACCAATTTCACACTTCTTGAGTTCTGCCGT				
LeIF H	CTTGCAAAAAGATTAAAGGAGGAAGGATTGAAAACCTGGTTTCATCATGGAAATGATTTTCATTTGACTAATACATCATCTCACACTTTTCATGTTCTTCCATT				
LeIF A	TCAAAGACTCATGTTTCTGCTATGACCATGACACGATTTAAATCTTTTCAAAATGTTTTAGGAGTATTAATCAACATTTGATTAACAGCTCTTAAGGCACTA				
LeIF B	TTTCAAAGACCCCTTGTTTTCTGCCAAAACCATGCTATGAATTTGAATCAAAATGTTCAAGTGTTCAGGAGTGTAAAGCAACATCCTGTTCAGCTGTATGG				
LeIF C	TTTCAAAGACTCACTTCTATAAACCACGACGCGTTGAATCAAAAATTTCAAATGTTTTCAGCAGTGTAAAGAGTGTGTTACCTGTGCAGGCACTAG				
LeIF D	TTTCAAAGACTCACCCCTGCTATAACTATGACCATGCTGATAAAGTATTAATTAATTAATTAATTAATTAATTAATTAATTAATTAATTAATTAATTAATTTT				
LeIF E	TGCCATTTCTCATTTTIGCTATAATCCATGACATGAGTTGAATCAAAAATTTAAAATGTTTTCAGGAATGTTAAGCAGCATCATGTTTCAGCTGTACAGGCA				
LeIF F	TTCAAAGACTCATTTCTCCTATAAACCACCGCATGAGTTGAATCAAAAATTTTCAGATCTTTTCAGGAGTGTAGGAAACATCATGTTTACCCTGTGCAGGCA				
LeIF G	TTCAAATATTAATTTCTGCTATAATCCATGACTTGAGTTGAATCAAAAATTTTCAAACGTTTCACACGTTAAGCAACACTTCTTTAGCTCCACAGGGACA				
LeIF H	TCAAAGACTCACTTCTATAAACCACCAAGTTGAATCAAAAATTTCCAAATGTTTTCAGGAGTGTAAAGAGCATCGTGTTCACCTGTGCAGGCACTAGTC				
	660	680	700	720	740

FIG. 3D

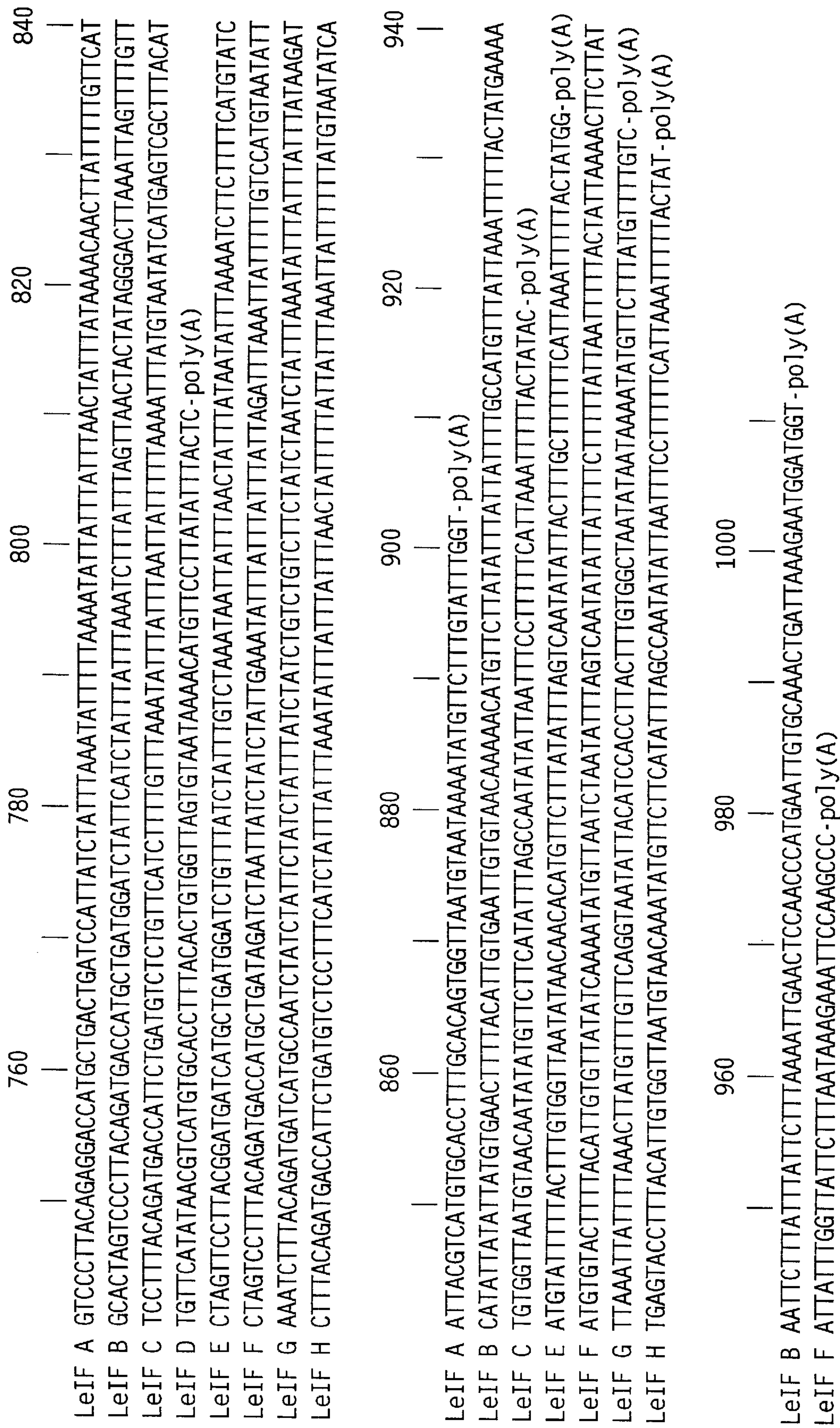


FIG. 3E

S1		S10		S20		S23		10		20		30		40			
LeIF A	MALTFALLVALLVLSCKSSCVGCDLPQTHSLGSRRTMLLAQMRKISLFSCLKDRHDFGFPPQ																
LeIF B	MALTFYLMVALVLSYKSFSSLGCDLPQTHSLGNRRRALILLAQMRRISPFSCCLKDRHDFEFPPQ																
LeIF C	MALSFSLLMAVLVLSYKICSISLGCDLPQTHSLGNRRRALILGQMGRISPFSCCLKDRHDFRIPQ																
LeIF D	MASPFALLMVLVLSCKSSCSLGCDLPETHSLDNRRRTMLLAQMSRISPFSSCLMDRHDFFGFPPQ																
LeIF E	LPLGCDLPQAHSVGNRRRAFILLTQMRRISPFSYLKDRHDFDFPH																
LeIF F	MALSFSLLMAVLVLSYKICSISLGCDLPQTHSLGNRRRALILLAQMGRISPFSCCLKDRHDFGFPPQ																
LeIF G	HDFGFPPQ																
LeIF H	MALPFSLLMALVLSCKSSCSLGCNLSQTHSLNRRRTMLMAQMRRISPFSCCLKDRHDFEFPPQ																
ATT	MA	F	L	VLS	KS	S	GC	L	THSL	RR	L	L	QM	IS	SCL	DRHDF	PQ

	50	60	70	80	90	100									
LeIF A	EEF-GNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNDLEACVIOG														
LeIF B	EEFDDKQFQKAQAISVLHEMIQQTFNLFSTKDSSAALDELLEFYIELDQQNDLEVLCDQE														
LeIF C	EEFDGNQFQKAQAISVLHEMIQQTFNLFSTEDSSAAWEQSLLEKSTELYQQNDLEACVIOE														
LeIF D	EEFDGNQFQKAPAI SVLHELIIQQIFNLFSTKDSSAAWDEDLDDKFCCTELYQQNDLEACVMQE														
LeIF E	QVFHGNHFQKVQAI FLFHEMMQQT FNLFSTKDSSDTWDETLLDKSYTELYQQNDLEACVM*K														
LeIF F	EEFDGNQFQKAQAISVLHEMIQQTFNLFSTKDSSATWEQSLLEKSTELNQQNDMEACVIOE														
LeIF G	EEFDGNQFQKAQAISVLHEMIQQTFNLFSTKDSSATWDETLLDKFYTELYQQNDLEACMMQE														
LeIF H	EEFDGNQFQKAQAISVLHEMMQQT FNLFSTKNSSAAWDETLLLEKFIYELFQQMNDLEACVIOE														
ATT	EEFD	QFQKA	I	VLHE	QQ	FNLE	T	SSA	LL	F	EL	QQ	ND	E	Q

FIG.4A

	110	120	130	140	150	160	166							
LeIF A	VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE													
LeIF B	VGVIESPMLMYEDSILAVRKYFQRITLYLTEKKYSSCAWEVVRAEIMRSFSLINLQKRLKSKE													
LeIF C	VGVEETPLMNEDSILAVRKYFQRITLYLIERKYSPCAWEVVRAEIMRSLSFSTNLQKRLRRKD													
LeIF D	ERVGETPLMNVDSILAVKKYFRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE													
LeIF E	VGVEETPLRNVDSILAVRKYFQRITLYLTKKKYSPCSWEAVRAEIMRSFSL*TNLQERLRRKE													
LeIF F	VGVEETPLMNVDSILAVKKYFQRITLYLTEKKYSPCAWEVVRAEIMRSFSLSKIFQERLRRKE													
LeIF G	VGVEDTPLMNVDSILTVRKYFQRITLYLTEKKYSPCAWEVVRAEIMRSFSLSANLQERLRRKE													
LeIF H	VGVEETPLMNEDSILAVRKYFQRITLYLMEKKYSPCAWEVVRAEIMRSFSLSTNLQKRLRRKD													
A11	V	PLM	DSI \bar{L}	V	KYF	RIT \bar{L} YL	E	KYS	CAWEVVRAEIMRS	S	S	Q	\bar{L}	K

FIG. 4B

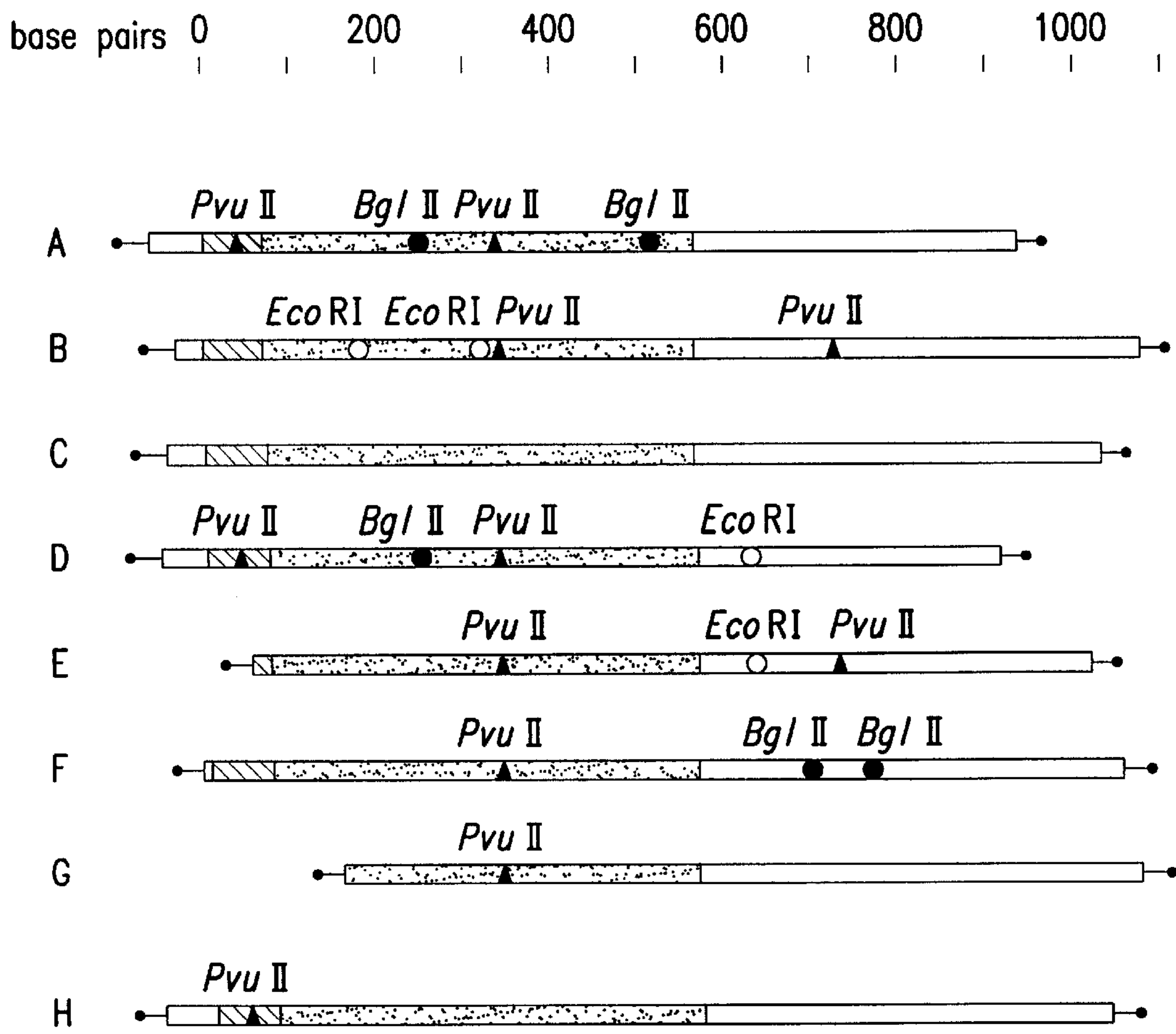


FIG.5

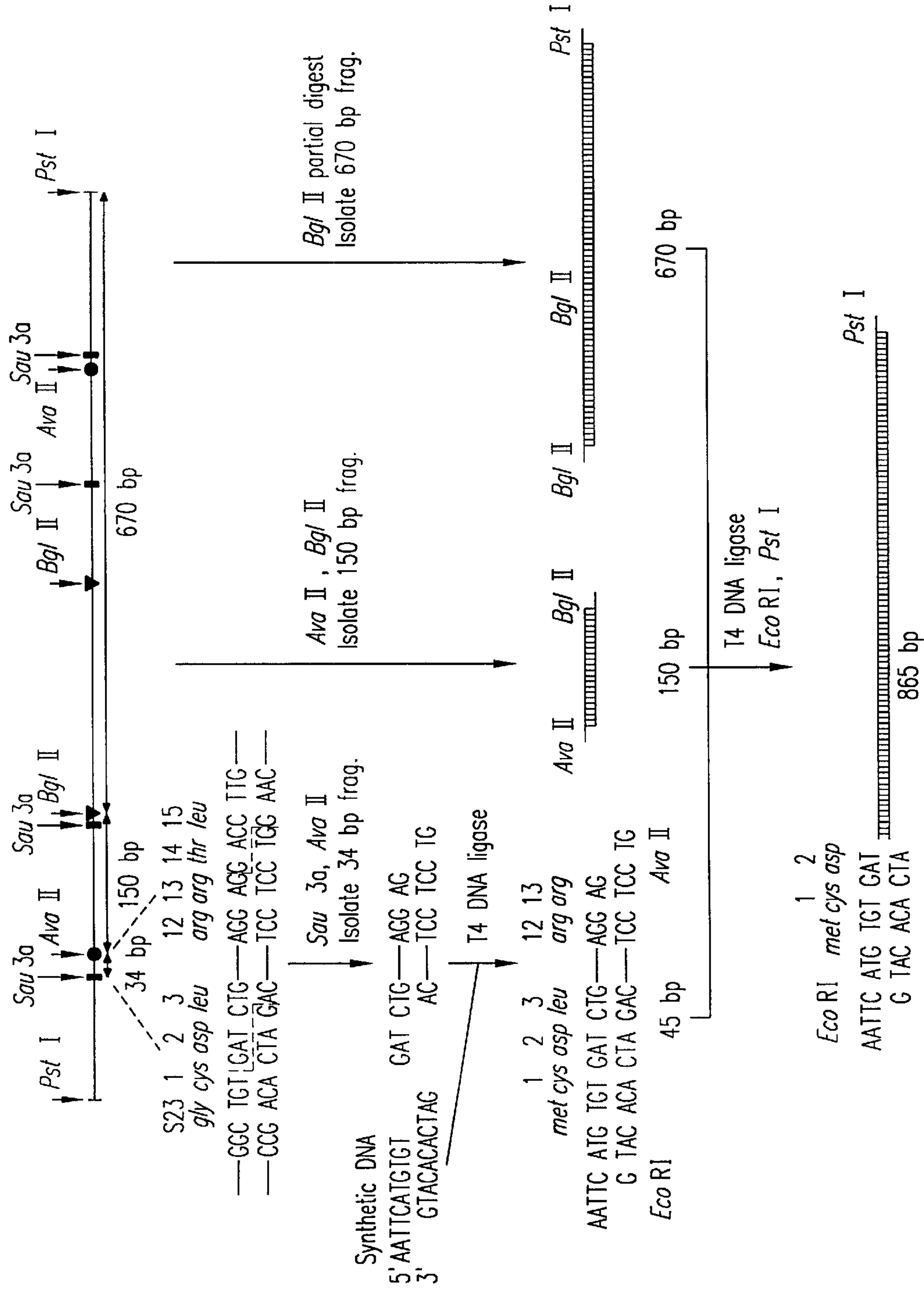


FIG. 6

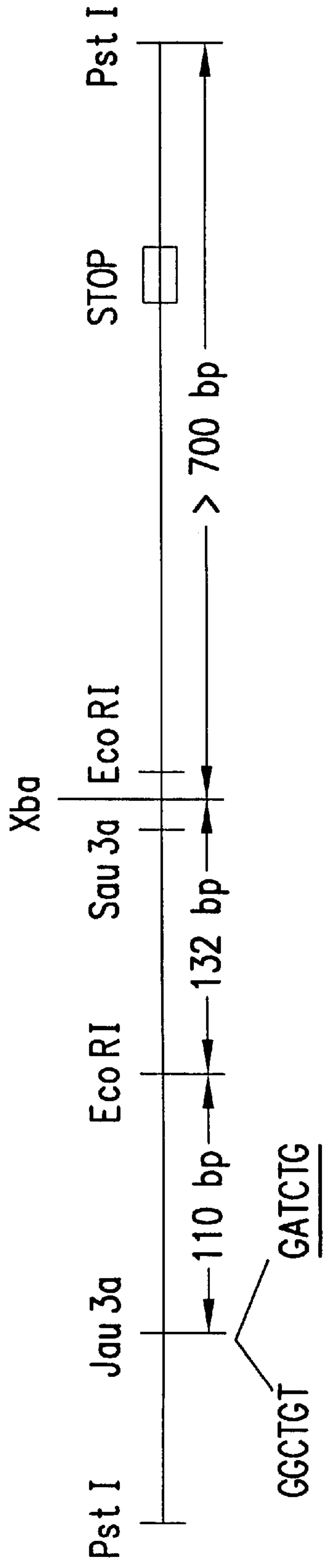


FIG. 7a

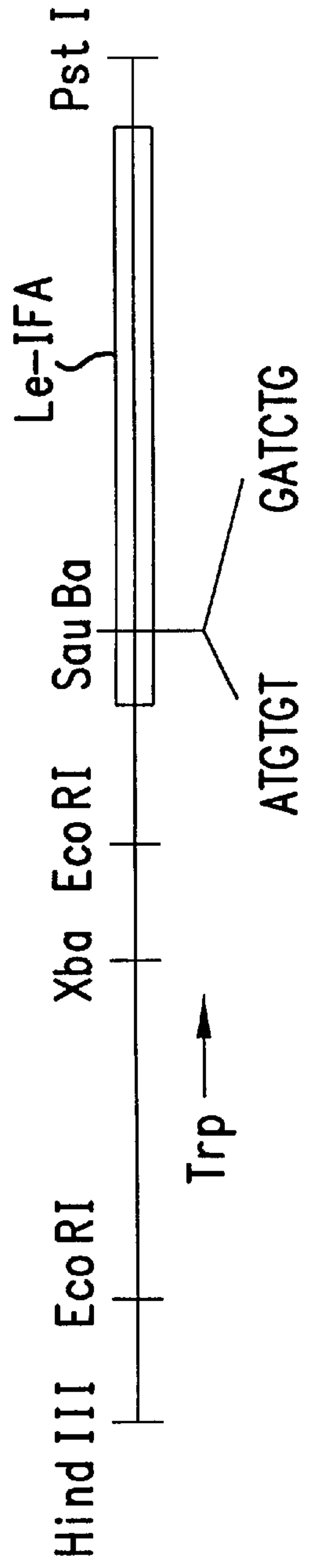


FIG. 7b

GTATGTTCCCTA
GTATGTTCCCTA
GTATGTTCCCTA
GTATGTTCACTA
GTATGTTCACTA

A
H
I
J
C

TTTAAGGC - TAGGCACAAGCAAGGCTTCAGAGAACCCTGGAGCCTAAGGTTTAGGCTCACCCATT - TCAACCAGTCTAGCAGCATCTGCAACATCTACA
TTTAAGGC - TAGGCACAAGCAAGGCTTCAGAGAACCCTGGAGCCTAAGGTTTAGGCTCACCCATT - TCAACCAGTCTAGCAGCATCTGCAACATCTACA
TTTAAGACCTATGCACAGAGCAAGGCTTCAGAAAACCTACAACCCAAAGGTCAGTGTACCCCTCATCAACCAGCCAGCAGCATCTTCAGGGTTCCCA
TTTAAGGCCTATGCACAGAGCAAGGCTTCAGAAAACCTAGAGGCCAAAGTTCAGAGTTACCCATC - TCAAGTAGCCTAGCAACATTTGCAACATCCCCA -
TTTAAGACCTATGCACAGAGCAAGGCTTCAGAAAACCTAGAGGCCACGGTTCAA - GTTACCCACC - TCAGGTAGCCTAGTGATAATTTGCAAAAATCCCCA -

A
H
I
J
C

+1
100
ATGGCCTTGACCTTTGCTTTACTGGTGGCCCTCCTGGTGCTCAGCTGCAAGTCAAGCTGCTGTGGGCTGTGATCTGCCTCAAACCCACAGCCTGGGTA
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ATGGCCCTGTCCCTTTCTTTACTGATGGCCGTGCTGGTGCTCAGCTACAAATCCATCTGTTCTTAGGCTGTGATCTGCCTCAGACCCACAGCCTGGGTA
ATGGCCCGGTCCCTTTCTTTACTGATGGTGTGCTGGTACTCAGCTACAAATCCATCTGCTCTCTGGGCTGTGATCTGCCTCAGACCCACAGCCTGCGTA
ATGGCCCTGTCCCTTTCTTTACTTATGGCCGTGCTGGTGCTCAGCTACAAATCCATCTGATCTCTGGGCTGTGATCTGCCTCAGACCCACAGCCTGCGTA

A
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200
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ATAGGAGGCCCTTGATACTCCTGGCACAAATGGGAAGAACTCTCCTTTCTCCTGCCTGAAGGACAGACCTGACTTTGGACTTTCCCAGGAGGATTTGA
ATAGGAGGCCCTTGATACTCCTGGCACAAATGGGAAGAACTCTCCTTTCTCCTGCTTGAAGGACAGACATGAAATTCAGATTTCCCAGAGGAGGATTTGA
ATAGGAGGCCCTTGATACTCCTGGCACAAATGGGAAGAACTCTCCTTTCTCCTGCCTGAAGGACAGACATGATTTCCGAAATCCCAGGAGGATTTGA

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J
C

FIG. 8A

300

A -GGCAACCAGTTCCAAAAGGCTGAAACCATCCCTGTCTCCATGAGATGATCCAGCAGATCTTCAATCTCTCAGCACAAAGGACTCATCTGCTGCTTGG
 H TGGCAACCAGTTCCAGAAAGCTCAAGCCATCTCTGTCTCCATGAGATGATGCAGCAGACCTTCAATCTCTCAGCACAAAGACTCATCTGCTGCTTGG
 I TGGCAACCAGTTCCAGAAGACTCAAGCCATCTCTGTCTCCATGAGATGATCCAGCAGACCTTCAATCTCTCAGCACAGAGGACTCATCTGCTGCTTGG
 J TGGCCACCAGTTCCAGAAGACTCAAGCCATCTCTGTCTCCATGAGATGATCCAGCAGACCTTCAATCTCTCAGCACAGAGGACTCATCTGCTGCTTGG
 C TGGCAACCAGTTCCAGAAGGCTCAAGCCATCTCTGTCTCCATGAGATGATCCAGCAGACCTTCAATCTCTCAGCACAGAGGACTCATCTGCTGCTTGG

400

A GATGAGACCCTCCTAGACAAAATTCTACACTGAACTACCAGCAGCTGAATGACCTGGAAGCCTGTGTGATACAGGGGTGGGGTGACAGAGACTCCCC
 H GATGAGACCCTCCTAGAAAAAATTCTACATTTGAACTTTTCCAGCAATGAATGACCTGGAAGCCTGTGTGATACAGGAGGTGGGGTGGAAAGAGACTCCCC
 I GAACAGAGCCTCCTAGAAAAAATTTCCACTGAACTTTACCAGCAACTGAAATAACCTGGAAGCATGTGTGATACAGGAGGTGGGATGGAAGAGACTCCCC
 J GAACAGAGCCTCCTAGAAAAAATTTCCACTGAACTTTACCAGCAACTGAAATGACCTGGAAGCATGTGTGATACAGGAGGTGGGGTGGAAAGAGACTCCCC
 C GAACAGAGCCTCCTACAAAAAATTTCCACTGAAAATTTACCAGCAACTGAAATGACCTGGAAGCATGTGTGATACAGGAGGTGGGGTGGAAAGAGACTCCCC

500

A TGATGAAGGAGGACTCCATTCGGCTGTGAGGAAATACATCCAAAGAAATCACTCTCTATCTGAAAGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTGT
 H TGATGAATGAGGACTCCATCCTGGCTGTGAGAAATACATCCAAAGAAATCACTCTTTATCTGATGGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTGT
 I TGATGAATGAGGACTCCATCCTGGCTGTGAGGAAATACATCCAAAGAAATCACTCTTTATCTAACAGAGAAGAAATACAGCCCTTACAGCCTGGGAGGTGT
 J TGATGAATGAGGACTTCATCCTGGCTGTGAGGAAATACATCCAAAGAAATCACTCTTTATCTAATGGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTGT
 C TGATGAATGAGGACTCCATCCTGGCTGTGAGGAAATACATCCAAAGAAATCACTCTTTATCTAATAGAGAGGAAATACAGCCCTTGTGCCTGGGAGGTGT

FIG. 8B

	600
A	CAGAGCAGAAATCATGAGATCTTTTCTTTGTCAACAACTTGCAAGAAGTTAAGAAGTAAGGAATGAAAACTGGTTCACATGGAAATGATTTTCAT
H	CAGAGCAGAAATCATGAGATCCCCTCTCTTTTCAACAACTTGCAAAAAGATTAGGAGGAAGGATTGAAAAGTGGTTCATCATGGAAATGATTTCTCAT
I	CAGAGCAGAAATCATGAGATCTCTCTCTTTTCAACAACTTGCAAAAATAATTAAGGAGGAAGGATTGAAAACCTGGTTCACATGGCAATGATCCTGAT
J	CAGAGCAGAAATCATGAGATCCCTTCTCTTTTCAACAACTTGAAAAGGATTAGGAGGAAGGATTGAAAACCTGGTTCATCATGGAAATGATTTCTCAT
C	CAGAGCAGAAATCATGAGATCCCCTCTCGTTTCAACAACTTGCAAAAAGATTAGGAGGAAGGATTGAAAACCTGGTTCACATGGCAATGATCCTGAT
	700
A	TGATTCGTATGCCAGCTCACCTTTTTATGATCTGCCATTTCAAGACTCATGTTTCTGCTATGACCATGACACGATTTAAATCTTTCAAATGTTTTTAG
H	TGACTAATACATCATCTCACACTTTCATGAGTTCTCCATTTCAAGACTCACCTTCTCCTATAACCCACCACAAGTTGAATCAAAATTTTCAAATGTTTTTC
I	TGACTAATACATTTATCTCACACTTTCATGAGTTCTCCATTTCAAGACTCACCTTCTATAACCCACCACGAGTTGAATCAAAATTTTCAAATGTTTTCAGC
J	TGACTAATGCATCATCTCACACTTTCATGAGTTCTCCATTTCAAGACTCACCTTCTATAACCCACCACAAGTTGAATCAAAATTTCCAAAATGTTTTCAGG
C	TGACTAATACATTTATCTCACACTTTCATGAGTTCTCCATTTCAAGACTCACCTTCTATAACCCACCACGAGTTGAATCAAAATTTCAAATGTTTTCAGC
	800
A	GAGTATTAATCAACATTTGATTCAGCTCTTAAGGCACTAGTCCCTTACAGAGGACCATGCTGACTGATCCATTAICTATTTAAATATTTTTAAATAATTA
H	AGGAGTGTAAGAAGCATCATGTATACCTGTGCAGGCAC TAGTCCCTTACAGATGACCATGCTGATGCTCCCTTCATCTATTTAATAATTTAATTT
I	AGTGTAAGAAGCGTCGTGTATACCTGTGCAGGCAC TAGTACTTTACAGATGACCATGCTGATGCTCTGTTCACTATTTAATTTAAATATTTAATTAAT
J	AGTGTTAAGAAGCATCGTGTTTACCTGTGCAGGCAC TAGTCCCTTACAGATGACCATTCIGATGCTCCCTTCATCTATTTAATTTAAATATTTAATTTAAT
C	AGTGTAAGAAGTGTGTTATACCTGTGCAGGCAC TAGTCCCTTACAGATGACCATTCIGATGCTCTGTTCACTCTTTGTTTAAATAATTTAATTTAAT

FIG. 8C

900

A TTTATTTAACTATTTATAAAACAACCTTATTTTTGGTTCATATTATGTCATGTGCACCTTTGCACAGTGGTAAATGTAATAAAATGIGTTCCTTTGTATTTGG
H ATTTAACTATTTTTATTTAAATTTAATTTTTAATGTTAATAATCATGTGACCTTTACATTTGGTTAAATAAACAAATATGTTCTTCATATTTAGCCAA
I TATTTTTAAGATTTAAATTTATTTTTATGTAATATCATGTGTACCTTTACATTTGGTGAATGTAACAATATATGTTCTTCATATTTAGCCAAATATATT
J TAACTATTTTTAATTTAAATTTAATTTTTATGTAATATCATATGTACCTTTACATTTGGTTAATGTAACAATAATGTTCTTCATATTTAGCCAAATATA
C ATTTTTAAATTTATGTAATATCATGAGTCGCTTTACATTTGGTTAATGTAACAATATATGTTCTTCATATTTAGCCAAATATATTAATTTCCCTTTTCA

1,000

A TAAATTTATTTTGGTGTTCATTTGAACCTTTTGCTATGGAACCTTTTGTACTTGTTTATTTCTTTAAATGAAATTTCCAAGCCTAATTTGTGCAACCTGATTA
H TATATAATTTCCCTTTTTCATTTAAATTTTTACTATACAAAATTTCTGTGTTGGTATTT
I AATTTCCCTTTTTCATTTAAATTTTACTATACAAAATTTCTTGAGTTTGTTTATTTCTTAAGAAATAAAATGTCGAGGCTGACTTTACAACCTGACTTAAAAA
J TTAATTTCCCTTTT CATTAAATTTTACTATACAAAATTTCTTGTGTTTGTATTTTTAAGATTTAAATGCCAAGCCTGACTGTATAACCTGACTTAA
C TTAATTTTACTATACAAAATTTCTTGTGTTTGTATTTCTTTAAGATAAAATGCCAAGCCTGACTTTACAACCTGACTTTAAAAATAGATGATTTAATT

FIG. 8D

	100		110		120
A	Q L N D L E A C V I Q G V G V T E T P L M K E D S I L A V R K Y F Q R I T L				
H	Q M N D L E A C V I Q E V G V E E T P L M N E D S I L A V R K Y F Q R I T L				
I	Q L N N L E A C V I Q E V G M E E T P L M N E D S I L A V R K Y F Q R I T L				
J	Q L N D L E A C V I Q E V G V E E T P L M N E D F I L A V R K Y F Q R I T L				
C	Q L N D L E A C V I Q E V G V E E T P L M N E D S I L A V R K Y F Q R I T L				

	130	140	150	160
A	Y L K E K K Y S P C A W E V V R A E I M R S F S L S T N L Q E S L R S K E			
H	Y L M E K K Y S P C A W E V V R A E I M R S L S F S T N L Q K R L R R K D			
I	Y L T E K K Y S P C A W E V V R A E I M R S L S F S T N L Q K I L R R K D			
J	Y L M E K K Y S P C A W E V V R A E I M R S F S F S T N L K K G L R R K D			
C	Y L I E R K Y S P C A W E V V R A E I M R S L S F S T N L Q K R L R R K D			

FIG. 9B

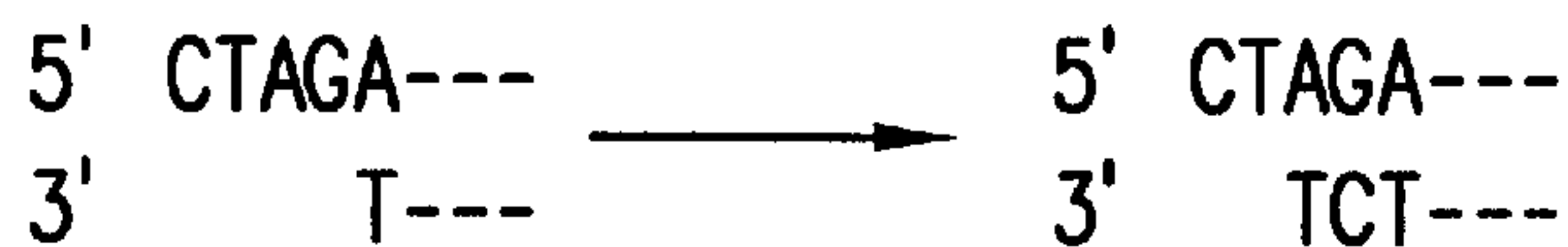


FIG.10

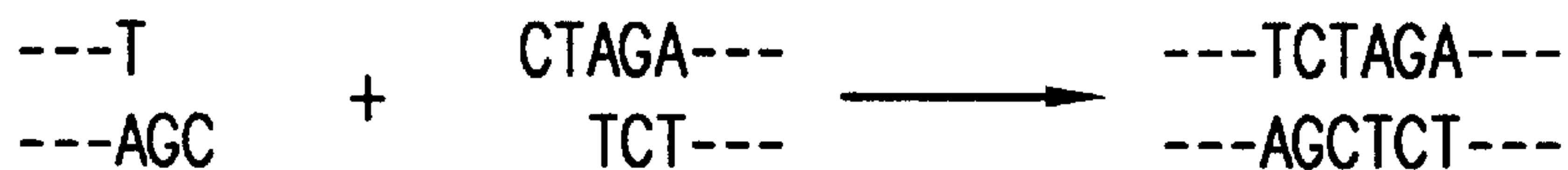


FIG.11

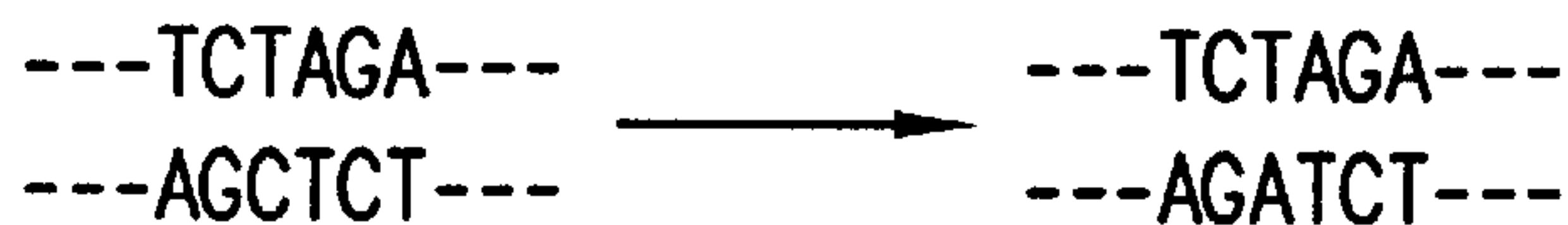


FIG.12

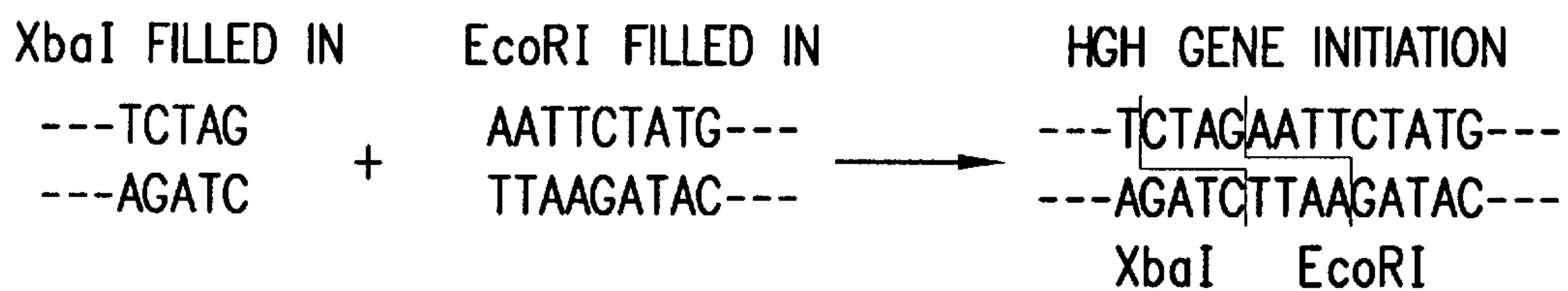


FIG.13

MICROBIAL PRODUCTION OF MATURE HUMAN LEUKOCYTE INTERFERONS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of application Ser. No. 06/703,148, filed Feb. 19, 1985, now abandoned, which is a division of application Ser. No. 06/256,204 filed Apr. 21, 1981, which is a continuation-in-part of application Ser. No. 06/205,578, filed Nov. 10, 1980, now abandoned, which is a continuation-in-part of application Ser. No. 06/184,909, filed Sep. 8, 1980, now abandoned, which is a continuation-in-part of application Ser. No. 06/164,986, filed Jul. 1, 1980, now abandoned.

FIELD OF THE INVENTION

This invention relates to the microbial production, via recombinant DNA technology, of human leukocyte interferons for use in the treatment of viral and neoplastic diseases, and to the means and end products of such production.

BACKGROUND OF THE INVENTION

The publications and other materials referred to herein to illuminate the background of the invention and, in particular cases, to provide additional detail respecting its practice are incorporated herein by reference and, for convenience, are numerically referenced in the following text and respectively grouped in the appended bibliography.

Leukocyte Interferon

Human leukocyte interferon was first discovered and prepared in the form of very crude precipitates by Isaacs and Lindenmann (3). Efforts to purify and characterize the material have been ongoing since that time, and have led to the preparation of relatively homogeneous leukocyte interferons derived from normal or leukemic (chronic myelogenous leukemia or "CML") donors' leukocytes (4). These interferons are a family of proteins characterized by a potent ability to confer a virus-resistant state in their target cells (1,2). In addition, interferon can act to inhibit cell proliferation and modulate immune response. These properties have prompted the clinical use of leukocyte interferon as a therapeutic agent for the treatment of viral infections and malignancies.

Leukocyte interferons have been purified to essential homogeneity (7,8), and reported molecular weights range from about 17,500 to about 21,000. The specific activity of these preparations is remarkably high, 2×10^8 to 1×10^9 units/mg protein, but yields from cell culture methods have been discouragingly low. Nevertheless, advances in protein sequencing techniques have, in our hands, permitted the determination of partial amino acid sequences (4). Elucidation of the glycosylation of various leukocyte interferons is not at present complete, but it is now clear (by virtue of the work reported infra) that differences in glycosylation among family members does not alone account for the spectrum of molecular weights observed. Instead, the leukocyte interferons differ markedly in amino acid composition and sequence, and amino acid homology is, in some cases, less than 80 percent.

While isolation from donor leukocytes has provided sufficient material for partial characterization and limited clinical studies with homogeneous leukocyte interferon, it is a totally inadequate source for the amounts of interferon needed for large scale clinical trials and for broad scale prophylactic and/or therapeutic use thereafter. Indeed, presently clinical investigations employing human leukocyte-

derived interferons in antitumor and antiviral testing have principally been confined to crude (<1 percent pure) preparations of the material, and long lead times for the manufacture of sufficient quantities, even at unrealistic price levels, have critically delayed investigation on an expanded front.

Recombinant DNA Technology

With the advent of recombinant DNA technology, the controlled microbial production of an enormous variety of useful polypeptides has become possible. Already in hand are bacteria modified by this technology to permit the production of such polypeptide products such as somatostatin (5), the (component) A and B chains of human insulin (9) and human growth hormone (18). More recently, recombinant DNA techniques have been used to occasion the bacterial production of proinsulin and thymosin alpha 1, an immune potentiating substance produced by the thymus.

Other workers have reported on the obtention of DNA coding for human leukocyte interferon and to resultant proteins having leukocyte interferon activity—cf. Nagata et al., *Nature* 284, 316 (1980); Mantei et al., *Gene* 10, 1 (1980). See also Taniguchi et al., *Nature* 285, 547 (1980).

The workhorse of recombinant DNA technology is the plasmid, a non-chromosomal loop of double-stranded DNA found in bacteria and other microbes, oftentimes in multiple copies per cell. Included in the information encoded in the plasmid DNA is that required to reproduce the plasmid in daughter cells (i.e., a "replicon") and ordinarily, one or more selection characteristics such as, in the case of bacteria, resistance to antibiotics which permit clones of the host cell containing the plasmid of interest to be recognized and preferentially grown in selective media. The utility of plasmids lies in the fact that they can be specifically cleaved by one or another restriction endonuclease or "restriction enzyme", each of which recognizes a different site on the plasmidic DNA. Thereafter heterologous genes or gene fragments may be inserted into the plasmid by endwise joining at the cleavage site or at reconstructed ends adjacent to the cleavage site. DNA recombination is performed outside the cell, but the resulting "recombinant" plasmid can be introduced into it by a process known as transformation and large quantities of the heterologous gene-containing recombinant plasmid obtained by growing the transformant. Moreover, where the gene is properly inserted with reference to portions of the plasmid which govern the transcription and translation of the encoded DNA message, the resulting expression vehicle can be used to actually produce the polypeptide sequence for which the inserted gene codes, a process referred to as expression. Expression is initiated in a region known as the promoter which is recognized by and bound by RNA polymerase. In some cases, as in the tryptophan or "trp" promoter preferred in the practice of the present invention, promoter regions are overlapped by "operator" regions to form a combined promoter-operator. Operators are DNA sequences which are recognized by so-called repressor proteins which serve to regulate the frequency of transcription initiation at a particular promoter. The polymerase travels along the DNA, transcribing the information contained in the coding strand from its 5' to 3' end into messenger RNA which is in turn translated into a polypeptide having the amino acid sequence for which the DNA codes. Each amino acid is encoded by a nucleotide triplet or "codon" within what may for present purposes be referred to as the "structural gene", i.e. that part which encodes the amino acid sequence of the expressed product. After binding to the promoter, the RNA polymerase first transcribes nucleotides encoding a ribosome binding site,

then a translation initiation or "start" signal (ordinarily ATG, which in the resulting messenger RNA becomes AUG), then the nucleotide codons within the structural gene itself. So-called stop codons are transcribed at the end of the structural gene whereafter the polymerase may form an additional sequence of messenger RNA which, because of the presence of the stop signal, will remain untranslated by the ribosomes. Ribosomes bind to the binding site provided on the messenger RNA, in bacteria ordinarily as the mRNA is being formed, and themselves produce the encoded polypeptide, beginning at the translation start signal and ending at the previously mentioned stop signal. The desired product is produced if the sequences encoding the ribosome binding site are positioned properly with respect to the AUG initiator codon and if all remaining codons follow the initiator codon in phase. The resulting product may be obtained by lysing the host cell and recovering the product by appropriate purification from other bacterial protein.

We perceived that application of recombinant DNA technology would be the most effective way of providing large quantities of leukocyte interferon which, despite the absence in material so produced of the glycosylation characteristic of human-derived material, could be employed clinically in the treatment of a wide range of viral and neoplastic diseases.

More particularly, we proposed and have since succeeded in producing mature human leukocyte interferon microbially, by constructing one or more genes therefor which could then be inserted in microbial expression vehicles and expressed under the control of microbial gene regulatory controls.

Our approach to obtaining a first leukocyte gene involved the following tasks:

1. Partial amino acid sequences would be obtained by characterization of leukocyte interferon purified to essential homogeneity, and construct sets of synthetic DNA probes constructed whose codons would, in the aggregate, represent all the possible combinations capable of encoding the partial amino acid sequences.

2. Bacterial colony banks would be prepared containing cDNA from induced messenger RNA. Other induced mRNA that had been radio-labelled would be hybridized to plasmid cDNA from this bank. Hybridizing mRNA would be eluted and tested for translation into interferon in oocyte assay. Plasmid DNA from colonies shown positive for interferon in this manner would be further tested for hybridization to probes made as described in (1) above.

3. Parallel to the approach in part (2) above, induced mRNA-derived cDNA in plasmids would be used to form an independent bank of transformant colonies. The probes of part (1) would be used to prime the synthesis of radio-labelled single stranded cDNA for use as hybridization probes. The synthetic probes would hybridize with induced mRNA as template and be extended by reverse transcription to form induced, radio-labelled cDNA. Clones from the colony bank that hybridized to radio-labelled cDNA obtained in this manner would be investigated further to confirm the presence of a full-length interferon encoding gene. Any partial length putative gene fragment obtained in parts (1) or (2) would itself be used as a probe for the full-length gene.

4. The full-length gene obtained above would be tailored, using synthetic DNA, to eliminate any leader sequence that might prevent microbial expression of the mature polypeptide and to permit appropriate positioning in an expression vehicle relative to start signals and the ribosome binding site of a microbial promoter. Expressed interferon would be purified to a point permitting confirmation of its character

and determination of its activity notwithstanding the absence of glycosylation.

5. The interferon gene fragment prepared in the foregoing fashion could itself be used in probing, by hybridization, for other partially homologous leukocyte interferon species.

BRIEF SUMMARY OF INVENTION

We have discovered and, through recombinant DNA technology, enabled the microbial production in high yield of the family of homologous leukocyte interferons (sans glycosylation) as mature polypeptides, essentially unaccompanied by the corresponding presequence or any portion thereof. These may be directly expressed, recovered and purified to levels fitting them for use in the treatment of viral and malignant diseases of animals and man. Family members so far expressed have proven efficacious in in vitro testing and, in the first such demonstration of its kind, in in vivo testing as well, the latter involving the first mature leukocyte interferon to have been microbially produced. The invention comprises the interferons so produced and means of producing them.

Reference herein to the expression of a "mature leukocyte interferon," connotes the bacterial or other microbial production of an interferon molecule unaccompanied by associated glycosylation and the presequence that (as we have discovered) immediately attends mRNA translation of a human leukocyte interferon genome. Mature leukocyte interferon, according to the present invention, is immediately expressed from a translation start signal (ATG) just before the first amino acid codon of the natural product, in which event the mature polypeptide includes the methionine for which ATG codes without essentially altering its character, or the microbial host may process the translation product to delete the initial methionine. Mature leukocyte interferon could be expressed together with a conjugated protein other than the conventional leader, the conjugate being specifically cleavable in an intra- or extracellular environment. See British Patent Publication No. 2007676A. Finally, the mature interferon could be produced in conjunction with a microbial "signal" peptide which transports the conjugate to the cell wall, where the signal is processed away and the mature polypeptide secreted.

Particular leukocyte interferon proteins hereof have been defined by means of determined DNA gene and deductive amino acid sequencing—cf. FIGS. 3, 4, 8 and 9, for example. It will be understood that for these particular interferons, indeed all of the family of leukocyte interferon proteins embraced herein, natural allelic variations exist and occur from individual to individual. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. For each leukocyte interferon protein hereof, labelled LeIF A, LeIF B . . . LeIF J, etc., such allelic variations are included within the scope of the label or term defining such, and thus, this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts two series of synthetic deoxynucleotides designated T-1 and T-13 designed to prime cDNA synthesis from leukocyte interferon ("Le-IF") mRNA. Amino acid sequences are given for peptide 1 and a portion of peptide 13 derived from a tryptic digest of human Le-IF β (4). All potential mRNA sequences coding for these peptides are shown, as are the corresponding DNA sequences. Here and throughout, the letters A, T, C, G and U respectively connote

the nucleotides containing the bases adenine, thymine, guanine, cytosine and uracil, and polynucleotides are depicted as reading from the 5' (left) in the 3' (right) direction and, where double stranded ("d.s.") DNA is depicted, vice-versa for the bottom or non-coding strand.

FIG. 2 is an autoradiogram showing hybridization of potential Le-IF plasmids with ³²P-labelled synthetic deoxyoligonucleotides.

FIGS. 3A-3E depict the nucleotide sequence (coding strand) of eight gene fragments isolated as candidates for use in the expression of leukocyte interferons, respectively designated "A" through "H". The ATG translational initiation codon and the termination triplet for each LeIF is underlined. The stop codons or termination triplets are followed by 3' untranslated regions. The included full length gene for Le-IF "A" is missing one codon found in the others depicted, as indicated in the third "A" line of FIG. 3B. 5' untranslated regions precede the leader sequences. As isolated, fragment "E" lacked the full presequence or leader, but included the entire gene for the putative mature Le-IF "E". Fragment G as isolated lacked the full coding sequence. The nucleotide sequence in the rows labeled "LeIF A" (SEQ ID NO:1) encodes a 188 amino acid translation product (SEQ ID NO:2). The nucleotide sequence in the rows labeled "LeIF B" (SEQ ID NO:3) encodes a 189 amino acid translation product (SEQ ID NO:4). The nucleotide sequence in the rows labeled "LeIF C" (SEQ ID NO:5) encodes a 189 amino acid translation product (SEQ ID NO:6). The nucleotide sequence in the rows labeled "LeIF D" (SEQ ID NO:7) encodes a 189 amino acid translation product (SEQ ID NO:8). The nucleotide sequence in the rows labeled "LeIF E" (SEQ ID NO:9) encodes fragments of a translation product: nucleotides 1-186 encode a 62 amino acid fragment (SEQ ID NO:10), nucleotides 190-234 encode a 15 amino acid fragment (SEQ ID NO:62), nucleotides 250-273 encode an 8 amino acid fragment (SEQ ID NO:63), nucleotides 277-294 encode a 6 amino acid fragment (SEQ ID NO:64), nucleotides 298-420 encode a 41 amino acid fragment (SEQ ID NO:65), and nucleotides 424-534 encode a 37 amino acid fragment (SEQ ID NO:66). The nucleotide sequence in the rows labeled "LeIF F" (SEQ ID NO:11) encodes a 189 amino acid translation product (SEQ ID NO:12). The nucleotide sequence in the rows labeled "LeIF G" (SEQ ID NO:13) encodes a partial amino acid translation product (SEQ ID NO:14). The nucleotide sequence in the rows labeled "LeIF H" (SEQ ID NO:15) encodes a 189 amino acid translation product (SEQ ID NO:16). Nucleotides 126-623 of SEQ ID NO:15 encode a 166 residue mature amino acid translation product (SEQ ID NO:56). The nucleotide sequence in the rows labeled "LeIF A" (SEQ ID NO:1) encodes a 188 amino acid translation product (SEQ ID NO:2). The nucleotide sequence in the rows labeled "LeIF B" (SEQ ID NO:3) encodes a 189 amino acid translation product (SEQ ID NO:4). The nucleotide sequence in the rows labeled "LeIF C" (SEQ ID NO:5) encodes a 189 amino acid translation product (SEQ ID NO:6). The nucleotide sequence in the rows labeled "LeIF D" (SEQ ID NO:7) encodes a 189 amino acid translation product (SEQ ID NO:8). The nucleotide sequence in the rows labeled "LeIF E" (SEQ ID NO:9) encodes fragments of a translation product: nucleotides 1-186 encode a 62 amino acid fragment (SEQ ID NO:10), nucleotides 190-234 encode a 15 amino acid fragment (SEQ ID NO:62), nucleotides 250-273 encode an 8 amino acid fragment (SEQ ID NO:63), nucleotides 277-294 encode a 6 amino acid fragment (SEQ ID NO:64), nucleotides 298-420 encode a 41 amino acid fragment (SEQ ID NO:65), and nucleotides

424-534 encode a 37 amino acid fragment (SEQ ID NO:66). The nucleotide sequence in the rows labeled "LeIF F" (SEQ ID NO:11) encodes a 189 amino acid translation product (SEQ ID NO:12). The nucleotide sequence in the rows labeled "LeIF G" (SEQ ID NO:13) encodes a partial amino acid translation product (SEQ ID NO:14). The nucleotide sequence in the rows labeled "LeIF H" (SEQ ID NO:15) encodes a 189 amino acid translation product (SEQ ID NO:16). Nucleotides 126-623 of SEQ ID NO:15 encode a 166 residue mature amino acid translation product (SEQ ID NO:56).

FIGS. 4A-4B are a comparison of eight LeIF protein sequences predicted from nucleotide sequences. The one letter abbreviations recommended by the IUPAC-IUB Commission on Biochemical Nomenclature are used: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine. The numbers refer to amino acid position (S refers to signal peptide). The dash in the 165 amino acid LeIF A sequence at position 44 is introduced to align the LeIF A sequence with the 166 amino acid sequences of the other LeIFs. The LeIF E sequence was determined by ignoring the extra nucleotide (position 187 of FIG. 3B) in its coding region. The asterisks indicate in-phase termination codons. The amino acid sequence in the rows labeled "LeIF A" is a 188 residue LeIF A polypeptide (SEQ ID NO:17) with an intact signal peptide. The amino acid sequence labeled S1-S23 is the signal peptide, and the amino acid sequence labeled 1-166 is the mature LeIF A polypeptide (SEQ ID NO:45).

The amino acid sequence in the rows labeled "LeIF B" is a 189 residue LeIF B polypeptide (SEQ ID NO:18) with a portion of a signal peptide. The amino acid sequence labeled S1-S23 is the signal peptide, and the amino acid sequence labeled 1-166 is the mature LeIF B polypeptide (SEQ ID NO:46).

The amino acid sequence in the rows labeled "LeIF C" is a 189 residue LeIF C polypeptide (SEQ ID NO:19) with an intact signal peptide. The amino acid sequence labeled S1-S23 is the signal peptide, and the amino acid sequence labeled 1-166 is the mature LeIF C polypeptide (SEQ ID NO:47). The amino acid sequence in the rows labeled "LeIF D" is a 189 residue LeIF D polypeptide (SEQ ID NO:20) with an intact signal peptide. The amino acid sequence labeled S1-S23 is the signal peptide, and the amino acid sequence labeled 1-166 is the mature LeIF D polypeptide (SEQ ID NO:48). The amino acid sequences in the rows labeled "LeIF E" are fragments of a LeIF E polypeptide including the amino acid sequence labeled S20-101 (SEQ ID NO:21), the amino acid sequence labeled 103-154 (SEQ ID NO:67), and the amino acid sequence labeled 156-166 (SEQ ID NO:68). The amino acid sequence in the rows labeled "LeIF F" is a 189 residue LeIF F polypeptide (SEQ ID NO:22) with an intact signal peptide. The amino acid sequence labeled S1-S23 is the signal peptide, and the amino acid sequence labeled 1-166 is the mature LeIF F polypeptide (SEQ ID NO:49). The amino acid sequence in the rows labeled "LeIF G" is a 133 residue fragment of a LeIF G polypeptide (SEQ ID NO:23).

The amino acid sequence in the rows labeled "LeIF H" is a 189 residue LeIF H polypeptide (SEQ ID NO:24) with an intact signal peptide. The amino acid sequence labeled S1-S23 is the signal peptide, and the amino acid sequence labeled 1-166 is the mature LeIF H polypeptide (SEQ ID NO:50). Amino acids common to all LeIFs (excluding the

pseudogene LeIF E) are also shown in the rows labeled "All". The underlined residues are amino acids which are also present in human fibroblast interferon.

FIG. 5 depicts restriction endonuclease maps of the eight types of LeIF cloned cDNAs (A through H). The hybrid plasmids were constructed by the dC:dG tailing method Goeddel, D. V. et al, *Nature* 287, 411-416 (1980). Therefore, the cDNA inserts can be excised using Pst I. The lines at the end of each cDNA insert represent the flanking homopolymeric dC:dG tails. The positions of Pvu II, Eco RI and Bgl II restriction sites are indicated. Shaded regions of the figure represent the coding sequences of mature LeIFs; the cross-hatched regions indicate signal peptide coding sequences; and the open regions show 3' and 5' noncoding sequences.

FIG. 6 schematically depicts the construction of a gene coding for the direct microbial synthesis of mature Le-IF A. Restriction sites and residues are as shown ("Pst I", etc.). The term "b.p." connotes "base pair."

FIGS. 7(a and b) (not to scale) schematically depicts a restriction map of two gene fragments employed in expressing the mature leukocyte interferon Le-IF B. The codon sequences indicated are the coding strand termini resulting from digestion with the restriction enzyme Sau 3a in the two cases shown.

FIGS. 8A-8D provide the DNA sequences of the five LeIF proteins hereof, including types I and J. The nucleotide sequence (SEQ ID NO:25) in the rows labeled "A" encodes a 188 amino acid translation product (SEQ ID NO:26). The nucleotide sequence (SEQ ID NO:27) in the rows labeled "H" encodes a 189 amino acid translation product (SEQ ID NO:28). Nucleotides 180-677 of SEQ ID NO:27 encode a mature 166 amino acid translation product (SEQ ID NO:58). The nucleotide sequence (SEQ ID NO:29) in the rows labeled "I" encodes a 189 amino acid translation product (SEQ ID NO:30). Nucleotides 182-679 of SEQ ID NO:29 encode a mature 166 amino acid translation product (SEQ ID NO:59). The nucleotide sequence (SEQ ID NO:31) in the rows labeled "J" encodes a 189 amino acid translation product (SEQ ID NO:32). The nucleotide sequence (SEQ ID NO:33) in the rows labeled "C" encodes two amino acid translation products. Nucleotides 110-166 of SEQ ID NO:33 encode a 19 amino acid translation product (SEQ ID NO:69), and nucleotides 170-676 of SEQ ID NO:33 encode a 169 amino acid translation product (SEQ ID NO:34). Nucleotides 179-676 of SEQ ID NO:33 encode a mature 166 amino acid translation product (SEQ ID NO:57).

FIGS. 9A-9B provide the amino acid (see FIGS. 4A-4B above for the corresponding one letter abbreviations) sequences of five LeIF proteins hereof. The asterisk indicates a termination codon and the hyphen, a deletion or gap in the sequence. The amino acid sequence in the rows labeled "A" is a 188 residue polypeptide (SEQ ID NO:35) with an intact signal peptide. The amino acid sequence labeled S1-S23 is the signal peptide, and the amino acid sequence labeled 1-166 is the mature polypeptide (SEQ ID NO:53).

FIG. 10 schematically depicts filling in of 2 of the 4 nucleotides complementary to the 5' protruding end of the XbaI cleavage site.

FIG. 11 schematically depicts ligation of the Taq I protruding end of the Eco RI-Taq I fragment to the XbaI remaining protruding end of the fragment from pHS32.

FIG. 12 schematically depicts regeneration of the XbaI site via *E. coli* catalyzed DNA repair and replication.

FIG. 13 schematically depicts blunt end ligation of an XbaI filled in fragment with an Eco RI filled in fragment to recreate both the XbaI and the Eco RI site.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. Microorganisms Employed

The work described involved use of two microorganisms: *E. coli* x1776, as described in (11), and *E. coli* K-12 strain 294 (end A, thi⁻, hsr⁻, hsm_k⁺), as described in (12). Each has been deposited with the American Type Culture Collection, which isolated at 12301 parklawn Drive, Rockville, Mo. 20852 respectively ATCC accession nos. 31537 and 31446. All recombinant DNA work was performed in compliance with applicable guidelines of the National Institutes of Health.

The invention, in its most preferred embodiments, is described with reference to *E. coli*, including not only strains *E. coli* x1776 and *E. coli* K-12 strain 294, defined above, but also other known *E. coli* strains such as *E. coli* B, or other microbial strains many of which are deposited and (potentially) available from recognized microorganism depository institutions, such as the American Type Culture Collection (ATCC)—cf. the ATCC catalogue listing. See also German Offenlegungsschrift 2644432. These other microorganisms include, for example, Bacilli such as *Bacillus subtilis* and other enterobacteriaceae among which can be mentioned as examples *Salmonella typhimurium* and *Serratia marcesans*, utilizing plasmids that can replicate and express heterologous gene sequences therein. Yeast, such as *Saccharomyces cerevisiae*, may also be employed to advantage as host organism in the preparation of the interferon proteins hereof by expression of genes coding therefor under the control of a yeast promoter. (See the copending U.S. patent application Ser. No. 06,237,913 of Hitzeman et al., filed Feb. 25, 1981, assignee Genentech, Inc. et al., which is incorporated herein by reference.)

B. Source of Le-IF mRNA

Le-IF mRNA may be obtained from human leukocytes, ordinarily those of patients with chronic myelogenous leukemia, that have been induced to produce interferon with Sendai or NDV virus, as described in (4). A particularly preferred source, and that used in the work reported herein, is a cell line designated KG-1 derived from a patient with acute myelogenous leukemia. The cell line, described by Koeffler, H. P. and Golde, D. W., *Science* 200, 1153 (1978), reference (15), grows readily in a culture medium comprising RPMI 1640 plus 10 FCS (heat-inactivated), 25 mM Hepes buffer and 50 µg/ml of gentamicin, and is subcultured 1 to 3 split two times a week. Cells may be frozen from the foregoing growth medium plus 10 dimethyl sulfoxide. KG-1 has been deposited with the American Type Culture Collection, ATCC accession no. CRL 8031.

C. Messenger RNA Purification from KG-1 Cells

KG-1 cells were induced to produce interferon (and leukocyte interferon mRNA) with Sendai or NDV following the procedure described by Rubinstein et al., *Proc. Natl. Acad. Sci. USA* 76, 640 (1979) and Familetti et al., *Methods in Enzymology*, (1981) (in press) and (4). Cells were harvested 5 hours after induction and RNA prepared by the guanidine thiocyanate-guanidine hydrochloride procedure (14). RNA from uninduced cells was isolated in the same manner. Oligo (dT)—cellulose chromatography and sucrose gradient ultracentrifugation was used to obtain the 12 S sucrose gradient fraction of poly (A) RNA as described (16,21). This mRNA had an interferon titer of 8000-10,000 units per microgram in the *Xenopus laevis* oocyte assay (6).

D. Preparation of Colony Banks Containing Le-IF cDNA Sequences

5 µg of mRNA was used to prepare double stranded cDNA by standard, procedures (17,18). The cDNA was size frac-

tionated by electrophoresis on a 6 polyacrylamide gel and 230 ng of material ranging in size from 500 to 1500 base pairs were recovered by electroelution. A 100 ng portion of this cDNA was tailed with deoxyC residues (19), annealed with 470 ng of plasmid pBR322 (20) which had been tailed

with deoxy G residues at the Pst I site, and used to transform *E. coli* X1776. Approximately 130 tetracycline resistant, ampicillin sensitive transformants were obtained per ng of cDNA.

In a second similar experiment, approximately 1000 tetracycline resistant, ampicillin sensitive *E. coli* 294 transformants were obtained per ng of cDNA. In this case size fractionated cDNA material ranging in size from 600 to 1300 b.p. was recovered by electroelution for deoxyC tailing.

E. Preparation of Synthetic Oligonucleotides

The amino acid sequences of several tryptic fragments of human leukocyte interferon were determined (4). This information permitted the design of synthetic deoxyoligonucleotides potentially complementary to different regions of LeIF mRNA. The two tryptic peptides T1 and T13 were selected because they had amino acid sequences requiring the synthesis of only 12 and 4 undecamers, respectively, to account for all possible coding sequences (FIG. 1). Four sets of deoxyoligonucleotide probes were synthesized for each sequence, containing either three (T-1A, B, C, D) or one (T-13A, B, C, D) oligonucleotide each. The indicated complementary deoxyoligonucleotides 11 bases long were chemically synthesized by the phosphotriester method (24). Four individual probes were prepared in the T-13 series. The twelve T-1 probes were prepared in four pools of three probes as shown in FIG. 1.

F. Isolation of Partial Le-IF Gene Fragment Containing Plasmid No. 104

Transformants of *E. coli* x1776 were screened by the colony hybridization procedure (27) using ³²P-labelled induced mRNA as probe. Unlabelled mRNA from uninduced cells was mixed with the probe at a ratio of 200 to 1 to compete with uninduced mRNA present in the ³²P-labelled preparation. Hybridization of labelled mRNA should occur preferentially to colonies containing induced sequences. Three classes of transformants were obtained. (1) 2-3 of the colonies hybridized to ³²P-mRNA very strongly, (2) 10 hybridized significantly less than class 1, and (3) the remainder gave no detectable hybridization signal. This 3rd class was eliminated from further screening.

The positive colonies were examined for the presence of interferon-specific sequences by an assay which depends upon hybridization of interferon mRNA specifically to plasmid DNA. Initially, 60 strong positive colonies (class 1) were grown individually in 100 ml of M-9 broth supplemented with tetracycline (20 µg/ml), diaminopimelic acid (100 µg/ml), thymidine (20 µg/ml), and d-biotin (1 µg/ml). Ten cultures were pooled and plasmid DNA was isolated from the six pools as described earlier (34,35). Ten µg of each plasmid DNA pool were cleaved with HindIII, denatured and covalently bound to DBM paper (36). One µg of purified mRNA from induced cells was hybridized to each filter. Unhybridized mRNA was removed by washing. The specifically hybridized mRNA was eluted and translated in *Xenopus laevis* oocytes. By this assay, all six pools were negative. Five pools of ten colonies each and one pool of nine colonies were made from 59 weakly positive colonies, (class 2) and plasmids were prepared from the pools and examined as above. Among the six pools tested, one (K10) hybridized to interferon mRNA at levels significantly above background levels each time it was tested. In order to

identify the specific interferon cDNA clone plasmid DNAs were prepared from the 9 colonies of pool K10 and examined individually. Two of the nine plasmids (No. 101 and No. 104) bound interferon mRNA well above background levels.

G. Preparation and Use of cDNA Probes Obtained by Synthetic Oligonucleotide Priming of Induced mRNA; Identification of Colonies pL 1-30

A rapid plasmid isolation procedure (22) was used to prepare 1 µg of plasmid DNA from each of 500 individual *E. coli* 294 transformants. Each DNA sample was denatured and applied to nitrocellulose filters in triplicate following a published procedure (23).

The four individual probes of the T-13 series and the twelve T-1 probes prepared in four pools of three primers each were used to prime the synthesis of radiolabelled single stranded cDNA for use as hybridization probes. The template mRNA was either the 12S RNA from Sendai-induced KG-1 cells (8000 units IF activity per µg) or total poly (A) mRNA from uninduced leukocytes (<10 units per µg). ³²P-labelled cDNA was prepared from these primers using published reaction conditions (25). The 60 µl reactions were performed in 20 mM Tris-HCl (pH8.3), 20 mM KCl, 8 mM MgCl₂, 30 mM β-mercaptoethanol. Reactions included one µg of each primer (i.e. 12 µg total for T-1 series, 4 µg total for T-13 series), 2 µg of "induced" 12S fraction mRNA (or 10 µg of uninduced poly (A) mRNA), 0.5 mM dATP, dGTP, dTTP, 200 µCi (α³²P)dCTP (Amersham, 2-3,000 Ci/mole), and 60 units reverse transcriptase (Bethesda Research Laboratories). Product was separated from unincorporated label by gel filtration on a 10 ml Sephadex G-50 column, treated with 0.3N NaOH for 30' at 70° C. to destroy RNA, and neutralized with HCl. Hybridizations were performed as described (23).

The three sets of nitrocellulose filters containing the 500 plasmid samples were hybridized with a) induced cDNA primed with the T-1 set of primers, b) T-13 primed induced cDNA, and c) uninduced cDNA prepared by using both sets of primers. Clones were considered positive if they hybridized more strongly to one or both of the induced cDNA probes than to the total uninduced probe. Thirty "positive" clones (pL1-pL30) were selected from the 500 for further analysis.

H. Selection of Additional "Positive" Colonies pL31-39 Using a Restriction Fragment of Plasmid 104

A unique 260 b.p. BglIII restriction fragment isolated from the plasmid 104 clone was labelled by a published procedure (26) with ³²P and used as probe to independently screen 400 *E. coli* 294 transformants by an in situ colony screening procedure (27). Nine colonies (pL31-pL39) were identified which hybridized to different extents with this probe. In addition, the labelled 260 bp fragment was used to independently screen 4000 *E. coli* 294 transformants in the same manner. 50 colonies were identified which hybridized to different extents with this probe. One contained the Le-IF G fragment, one contained the Le-IF H fragment, and one contained a fragment designated Le-IF H1, an apparent allele of Le-IF H. The hybrid plasmids which result are designated "pLe-IF H", etc.

I. Isolation and DNA Sequencing of a First Full-Length Le-IF Gene Fragment from pL1-39

Plasmid DNA was prepared from all 39 potential Le-IF cDNA clones and rescreened with the same 260 b.p. DNA probe using the dot hybridization procedure (23). Three plasmids (pL4, pL31, pL34) gave very strong hybridization signals, four (pL13, pL30, pL32, pL36) hybridized moderately, and three (pL6, pL8, pL14) hybridized weakly with the probe.

The 39 potential Le-IF cDNA recombinant plasmids were also screened by using ^{32}P -labelled synthetic undecamers (individual T-1 primer pools or individual T-13 primers) directly as hybridization probes. The hybridization conditions were chosen such that perfect base pairing should be required for detectable hybridization signals (28). Thus, plasmid DNA from the 39 clones was prepared by a standard cleared lysate procedure (29) and purified by Biorad Agarose A-50 column chromatography. Samples (3 μg) of each prep were linearized by treatment with Eco RI, denatured in alkali and spotted on 2 separate nitrocellulose filters (1.5 μg per spot) (23). Individual synthetic deoxyoligonucleotide primers and primer pools were phosphorylated with ($\gamma^{32}\text{P}$) ATP as follows: 50 pmoles of oligonucleotide and 100 pmoles of ($\gamma^{32}\text{P}$)ATP (New England Nuclear, 2500 Ci/mole) were combined in 30 μl of 60 mM Tris-HCl (pH8), 10 mM MgCl_2 , 15 mM β -mercaptoethanol. 2 units of T4 polynucleotide kinase were added and, after 30' at 37° C., ^{32}P labelled primers were purified by chromatography on 10 ml Sephadex G-50 columns. Hybridizations were performed using 10^6 cpm of primer T-13C or 3×10^6 cpm of primer pool T-1C. The hybridizations were performed at 15° C. for 14 hours in 6 \times SSC, 10 \times Denhardt's solution, as described by Wallace et al. (28). Filters were washed for 5' (3 times) at 0° C. in 6 \times SSC, dried, and exposed to x-ray film. Results are shown in FIG. 2 for ^{32}P primer pool T-13C and primer T-1C.

Plasmid DNA from clone 104 was found to give significant hybridization with primer pool T-1C and primer T-13C, but no detectable hybridization with the other undecamers. As shown in FIG. 2, several of the 39 potential Le-IF plasmids (pL2, 4, 13, 17, 20, 30, 31, 34) also hybridized with both of these probes. Restriction analysis showed that only one of these plasmids, pL31, also contained a 260 b.p. internal Bgl II fragment. Pst I digestion of pL31 showed the size of the cDNA insert to be approximately 1000 base pairs.

The entire Pst I insert of pL31 was sequenced by both the Maxam-Gilbert chemical method (30) and by dideoxy chain termination procedure (31) after subcloning Sau 3a fragments into an M13 vector. The DNA sequence is shown ("A") in FIGS. 3A-3E. The appropriate translational reading frame could be predicted from protein sequence information in hand (4), the known range of Le-IF molecular weights and the relative incidence of stop triplets in the three possible reading frames, and that in turn permitted prediction of the entire Le-IF amino acid sequence, including a pre- or signal peptide. The first ATG translational initiation codon is found 60 nucleotides from the 5' end of the sequence and is followed, 188 codons later, by a TGA termination triplet; there are 342 untranslated nucleotides at the 3' end followed by a poly (A) sequence. The putative signal peptide (presumably involved in the secretion of the mature LeIF from leukocytes) is 23 amino acids long. The 165 amino acids constituting the mature LeIF have a calculated MW of 19,390. We have termed the Le-IF encoded by pL31 "Le-IF A." It can be seen from the sequence data ("A") in FIG. 5 that tryptic peptides T1 and T13 of Le-IF B (4) (FIG. 1) correspond to amino acids 145-149 and 57-61 respectively of the Le-IF A. The actual DNA coding sequences found in these two regions are those represented by primer pool T1-C and primer T13-C as the data shown in FIG. 2 suggested.

J. Direct Expression of a First Mature Leukocyte Interferon 1. Generally
The procedure followed to express Le-IF A directly as a mature interferon polypeptide was a variant of that earlier employed for human growth hormone (18), insofar as it involved the combination of synthetic (N-terminal) and complementary DNAs.

As shown in FIG. 6, a Sau 3a restriction endonuclease site is conveniently located between codons 1 and 2 of Le-IF A. Two synthetic deoxyoligonucleotides were designed which incorporate an ATG translational initiation codon, restore the codon for amino acid 1 (cysteine), and create an Eco RI sticky end. These oligomers were ligated to a 34 b.p. Sau 3a-Ava II fragment of pL31. The resulting 45 b.p. product was ligated to two additional DNA fragments to construct an 865 base pair synthetic-natural hybrid gene which codes for Le-IF A and which is bounded by Eco RI and Pst I restriction sites. This gene was inserted into pBR322 between the Eco RI and Pst I sites to give the plasmid pLe-IF A1.

Plasmid pGM1 carries the *E. coli* tryptophan operon containing the deletion ΔLE1413 (G. F. Miozzari, et al., (1978) *J. Bacteriology* 133, 1457-1466) and hence expresses a fusion protein comprising the first 6 amino acids of the trp leader and approximately the last third of the trp E polypeptide (hereinafter referred to in conjunction as LE'), as well as the trp D polypeptide in its entirety, all under the control of the trp promoter-operator system. The plasmid, 20 μg , was digested with the restriction enzyme PvuII which cleaves the plasmid at five sites. The gene fragments were next combined with EcoRI linkers (consisting of a self complementary oligonucleotide of the sequence: pCATGAATTCATG) (SEQ ID NO:39) providing an EcoRI cleavage site for a later cloning into a plasmid containing an EcoRI site. The 20 μg of DNA fragments obtained from pGM1 were treated with 10 units T_4 DNA ligase in the presence of 200 pico moles of the 5'-phosphorylated synthetic oligonucleotide pCATGAATTCATG and in 20 μl T_4 DNA ligase buffer (20 mM tris, pH 7.6, 0.5 mM ATP, 10 mM MgCl_2 , 5 mM dithiothreitol) at 4° C. overnight. The solution was then heated 10 minutes at 70° C. to halt ligation. The linkers were cleaved by EcoRI digestion and the fragments, now with EcoRI ends were separated using 5 percent polyacrylamide gel electrophoresis (hereinafter "PAGE") and the three largest fragments isolated from the gel by first staining with ethidium bromide, locating the fragments with ultraviolet light, and cutting from the gel the portions of interest. Each gel fragment, with 300 microliters 0.1 \times TBE, was placed in a dialysis bag and subjected to electrophoresis at 100 v for one hour in 0.1 \times TBE buffer (TBE buffer contains: 10.8 gm tris base, 5.5 gm boric acid, 0.09 gm Na_2EDTA in 1 liter H_2O). The aqueous solution was collected from the dialysis bag, phenol extracted, chloroform extracted and made 0.2 M sodium chloride, and the DNA recovered in water after ethanol precipitation. The trp promoter-operator-containing gene with EcoRI sticky ends was identified in the procedure next described, which entails the insertion of fragments into a tetracycline sensitive plasmid which, upon promoter-operator insertion, becomes tetracycline resistant.

Plasmid pBRH1 (R. I. Rodriguez, et al., *Nucleic Acids Research* 6, 3267-3287 [1979]) expresses ampicillin resistance and contains the gene for tetracycline resistance but, there being no associated promoter, does not express that resistance. The plasmid is accordingly tetracycline sensitive. By introducing a promoter-operator system in the EcoRI site, the plasmid can be made tetracycline resistant.

pBRH1 was digested with EcoRI and the enzyme removed by phenol extraction followed by chloroform extraction and recovered in water after ethanol precipitation. The resulting DNA molecule was, in separate reaction mixtures, combined with each of the three DNA fragments obtained above and ligated with T_4 DNA ligase as previously described. The DNA present in the reaction mixture was used to transform competent *E. coli* K-12 strain 294, K.

Backman et al., Proc Nat'l Acad Sci USA 73, 4174-4198 [1976]) by standard techniques (V. Hershfield et al., Proc Nat'l Acad Sci USA 71, 3455-3459 [1974]) and the bacteria plated on LB plates containing 20 $\mu\text{g}/\text{ml}$ ampicillin and 5 $\mu\text{g}/\text{ml}$ tetracycline. Several tetracycline-resistant colonies were selected, plasmid DNA isolated and the presence of the desired fragment confirmed by restriction enzyme analysis. The resulting plasmid is designated pBRHtrp.

An EcoRI and BamHI digestion product of the viral genome of hepatitis B was obtained by conventional means and cloned into the EcoRI and BamHI sites of plasmid pGH6 (D. V. Goeddel et al., Nature 281, 544 [1979]) to form the plasmid pHS32. Plasmid pHS32 was cleaved with XbaI, phenol extracted, chloroform extracted and ethanol precipitated. It was then treated with 1 μl *E. coli* polymerase I, Klenow fragment (Boehringer-Mannheim) in 30 μl polymerase buffer (50 mM potassium phosphate pH 7.4, 7 mM MgCl_2 , 1 mM β -mercaptoethanol) containing 0.1 mM dTTP and 0.1 mM dCTP for 30 minutes at 0° C. then 2 hr. at 37° C. This treatment causes 2 of the 4 nucleotides complementary to the 5' protruding end of the XbaI cleavage site to be filled in. See FIG. 10.

Two nucleotides, dC and dT, were incorporated giving an end with two 5' protruding nucleotides. This linear residue of plasmid pHS32 (after phenol and chloroform extraction and recovery in water after ethanol precipitation) was cleaved with EcoRI. The large plasmid fragment was separated from the smaller EcoRI-XbaI fragment by PAGE and isolated after electroelution. This DNA fragment from pHS32 (0.2 μg), was ligated, under conditions similar to those described above, to the EcoRI-Taq I fragment of the tryptophan operon (~0.01 μg), derived from pBRHtrp.

In the process of ligating the fragment from pHS32 to the Eco RI-Taq I fragment, as described above, the Taq I protruding end is ligated to the XbaI remaining protruding end even though it is not completely Watson-Crick base-paired. See FIG 11.

A portion of this ligation reaction mixture was transformed into *E. coli* 294 cells, heat treated and plated on LB plates containing ampicillin. Twenty-four colonies were selected, grown in 3 ml LB media, and plasmid isolated. Six of these were found to have the XbaI site regenerated via *E. coli* catalyzed DNA repair and replication. See FIG. 13.

These plasmids were also found to cleave both with EcoRI and HpaI and to give the expected restriction fragments. One plasmid, designated pTrp 14, was used for expression of heterologous polypeptides, as next discussed.

The plasmid pGH 107 (D. V. Goeddel et al, Nature, 281, 544, 1979) contains a gene for human growth hormone made up of 23 amino acid codons produced from synthetic DNA fragments and 163 amino acid codons obtained from complementary DNA produced via reverse transcription of human growth hormone messenger RNA. This gene, though it lacks the codons of the "pre" sequence of human growth hormone, does contain an ATG translation initiation codon. The gene was isolated from 10 μg pGH 107 after treatment with EcoRI followed by *E. coli* polymerase I Klenow fragment and dTTP and dATP as described above. Following phenol and chloroform extraction and ethanol precipitation the plasmid was treated with BamHI.

The human growth hormone ("HGH") gene-containing fragment was isolated by PAGE followed by electroelution. The resulting DNA fragment also contains the first 350 nucleotides of the tetracycline resistance structural gene, but lacks the tetracycline promoter-operator system so that, when subsequently cloned into an expression plasmid, plasmids containing the insert can be located by the restoration of

tetracycline resistance. Because the EcoRI end of the fragment has been filled in by the Klenow polymerase I procedure, the fragment has one blunt and one sticky end, ensuring proper orientation when later inserted into an expression plasmid.

The expression plasmid pTrp14 was next prepared to receive the HGH gene-containing fragment prepared above. Thus, pTrp14 was XbaI digested and the resulting sticky ends filled in with the Klenow polymerase I procedure employing dATP, dTTP, dGTP and dCTP. After phenol and chloroform extraction and ethanol precipitation the resulting DNA was treated with BamHI and the resulting large plasmid fragment isolated by PAGE and electroelution. The pTrp14-derived fragment had one blunt and one sticky end, permitting recombination in proper orientation with the HGH gene containing fragment previously described.

The HGH gene fragment and the pTrp14 ΔXba -BamHI fragment were combined and ligated together under conditions similar to those described above. The filled in XbaI and EcoRI ends ligated together by blunt end ligation to recreate both the XbaI and the EcoRI site. See FIG. 13.

This construction also recreates the tetracycline resistance gene. Since the plasmid pGH 107 expresses tetracycline resistance from a promoter lying upstream from the HGH gene (the lac promoter), this construction, designated pGH 207, permits expression of the gene for tetracycline resistance under the control of the tryptophan promoter-operator. Thus the ligation mixture was transformed into *E. coli* 294 and colonies selected on LB plates containing 5 $\mu\text{g}/\text{ml}$ tetracycline.

Plasmid pGH207 was Eco RI digested and the trp promoter containing a 300 b.p. Eco RI fragment recovered by PAGE followed by electroelution. The 300 b.p. Eco RI fragment contains the *E. coli* trp promoter, operator, and trp leader ribosome binding site but lacks an ATG sequence for initiation of translation. This DNA fragment was cloned into the Eco RI site of pLe-IF A. The construction of the fragment is described in detail in (36).

2. The Tryptophan Control Element

The trp fragment just referred to is an analog of the *E. coli* tryptophan operon from which the so-called trp attenuator has been deleted, See (36), to controllably heighten expression levels. Expression plasmids containing the modified trp regulon can be grown to predetermined levels in nutrient media containing additive tryptophan in quantities sufficient to repress the promoter-operator system, then be deprived of tryptophan so as to derepress the system and occasion the expression of the intended product (36).

3. Detailed Description

More particularly, and with reference to FIG. 6, 250 μg of plasmid pL31 were digested with Pst I and the 1000 b.p. insert isolated by gel electrophoresis on a 6 polyacrylamide gel. Approximately 40 μg of insert was electroeluted from the gel and divided into 3 aliquots for further digestion: a) A 16 μg sample of this fragment was partially digested with 40 units of Bgl II for 45' at 37° C. and the reaction mixture purified on a 6 polyacrylamide gel. Approximately 2 μg of the desired 670 b.p. fragment were recovered. b) Another sample (8 μg) of the 1000 b.p. Pst I insert was restricted with Ava II and Bgl II. One μg of the indicated 150 b.p. fragment was recovered after gel electrophoresis. c) 16 μg of the 1000 b.p. piece was treated with Sau 3a and Ava II. After electrophoresis on a 10 polyacrylamide gel, approximately 0.25 μg (~10 pmole) of the 34 b.p. fragment was recovered. The two indicated deoxyoligonucleotides, 5'-dAATTCATGTGT (SEQ ID NO:42) (fragment 1) and 5'-d GATCACACATG (SEQ ID NO:43) (fragment 2) were

synthesized by the phosphotriester procedure (24). Fragment 2 was phosphorylated as follows. 200 μ l (~40 pmole) of (γ ³²P) ATP (Amersham, 5000 Ci/mmol) was dried down and resuspended in 30 μ l of 60 mM Tris-HCl (pH8), 10 mM MgCl₂, 15 mM β -mercaptoethanol, containing 100 pmoles of DNA fragment and 2 units of T4 polynucleotide kinase. After 15 minutes at 37° C., 1 μ l of 10 mM ATP was added and the reaction allowed to proceed another 15 minutes. The mixture was then heated at 70° C. for 15 minutes, combined with 100 pmole of 5'-OH fragment 1 and 10 pmole of the 34 b.p. Sau 3a-Ava II fragment. Ligation was performed for 5 hours at 4° C. in 50 μ l of 20 mM Tris-HCl (pH7.5) 10 mM Mg Cl₂, 10 mM dithiothreitol, 0.5 mM ATP and 10 units T4 DNA ligase. The mixture was electrophoresed on a 6 polyacrylamide gel and the 45 b.p. product recovered by electroelution. 860,000 Cerenkov cpm were recovered (~30 ng, 1 pmole), combined with 0.5 μ g (5 pmoles) of the 150 b.p. Ava II-Bgl II fragment and 1 μ g (2 pmoles) of the 670 b.p. Bgl II-Pst I fragment. The ligation was performed at 20° C. for 16 hours using 20 units of T4 DNA ligase. The ligase was inactivated by heating to 65° C. for 10 minutes. The mixture was then digested with Eco RI and Pst I to eliminate polymers of the gene. The mixture was purified by 6 percent polyacrylamide gel electrophoresis. 36,000 cpm (~0.04 pmole, 20 ng) of 865 b.p. product were isolated. One-half (10 ng) of this was ligated into pBR322 (0.3 μ g) between the Eco RI and Pst I sites. Transformation of *E. coli* 294 gave 70 tetracycline resistant, ampicillin sensitive transformants. Plasmid DNA isolated from 18 of these transformants was digested with Eco RI and Pst I. 16 of the 18 plasmids had an Eco RI-Pst I fragment 865 b.p. in length. One μ g of one of these, pLe-IF A1, was digested with Eco RI and ligated to a 300 b.p. Eco RI fragment (0.1 μ g.) containing the *E. coli* trp promoter and trp leader ribosome binding site, prepared as described above. Transformants containing the trp promoter were identified using a ³²P-trp probe in conjunction with the Grunstein-Hogness colony screening procedure (27). An asymmetrically located Xba I site in the trp fragment allowed determination of recombinants in which the trp promoter was oriented in the direction of the Le-IF A gene.

K. Induction of Interferon Expression and In Vitro Assay

Extracts were prepared for IF assay as follows. One ml cultures were grown in L broth containing 5 μ g/ml tetracycline to an A₅₅₀ of about 1.0, then diluted into 25 ml of M9 media containing 5 μ g/ml tetracycline. 10 ml samples were harvested by centrifugation when A₅₅₀ reached 1.0 and cell pellets were suspended in 1 ml of 15 percent sucrose, 50 mM Tris-HCl (pH 8.0), 50 mM EDTA. One mg of lysozyme was added and, after 5 minutes at 0° C., cells were disrupted by sonication. The samples were centrifuged 10 minutes at 15,000 rpm in a Sorvall SM-24 rotor. Interferon activity in the supernatants was determined by comparison with Le-IF standards by the cytopathic effect (CPE) inhibition assay (2). To determine the number of IF molecules per cell a Le-IF specific activity of 4 \times 10⁸ units/mg was used (7).

As shown in Table 1, Clone pLe-IF A trp 25, in which the trp promoter was inserted in the desired orientation, gives high levels of activity (as high as 2.5 \times 10⁸ units per liter). As shown in Table 2, the IF produced by *E. coli* K-12 strain 294/pLe-IF A trp 25 behaves like authentic human Le-IF; it is stable to treatment at pH2 and is neutralized by rabbit anti-human leukocyte antibodies. The interferon has an apparent molecular weight of approximately 20,000.

L. In Vivo Antiviral Activity of Le-IF A

The in vivo efficacy of interferon requires the presence of macrophages and NK cells and the in vivo mode of action appears to involve stimulation of these cells (33). Thus, it

remained possible that the interferon produced by *E. coli* 294/pLe-IF A25, while having antiviral activity in the cell culture assay, would not be active in infected animals. Moreover, the in vivo antiviral activity of the bacterially produced, non-glycosylated Le-IF A might be different from the glycosylated Le-IF derived from human "buffy coat" leukocytes. Therefore the biological activity of bacterially synthesized Le-IF A (~2 Pure) was compared with buffy coat Le-IF (~8 percent pure) in lethal encephalomyocarditis (EMC) virus infection of squirrel monkeys (Table 3).

TABLE 1

Interferon activity in extracts of <i>E. coli</i>			
<i>E. coli</i> K-12 strain 294 transformed by:	Cell density (cells/ml)	IF Activity units/ml culture	Le-IF molecules per cell
pLe-IF A trp 25	3.5 \times 10 ⁸	36,000	9,000
pLe-IF A trp 25	1.8 \times 10 ⁹	250,000	12,000

TABLE 2

	Comparison of activities of extracts from <i>E. coli</i> 294/pLe-IF A25 with standard Le-IF		
	Interferon Activity (units/ml)		
	untreated	pH2	rabbit anti-human leukocyte antibodies
294/pLeIF-A trp 25 extract	500	500	<10
Le-IF standard	500	500	<10

TABLE 3

Antiviral effect of various Le-IF preparations against EMC virus infection of squirrel monkeys				
Treatment	Survivors	Serum p.f.u./ml.		
		day 2	day 3	day 4
Control (bacterial proteins)	0/3	10 } 3	3 \times 10 ⁴ } 10 ⁴	>10 ⁵ } >3.4 \times 10 ⁴
Bacterial Le-IF A	3/3	0	0	0
Le-IF standard	3/3	0	0	0

All monkeys were male (average weight 713 g) and had no EMC virus antibodies prior to infection. The monkeys were infected intramuscularly with 100 \times LD₅₀ EMC virus (determined in mice). The control treated monkeys died at 134, 158 and 164 hours post-infection. Interferon treatments of 10⁶ units were by the intravenous route at -4, +2, 23, 29, 48, 72, 168 and 240 hours, relative to infection. The bacterial leukocyte interferon was a column chromatography fraction from a lysate of *E. coli* 294/pLe-IF A25 at a specific activity of 7.4 \times 10⁶ U/mg protein. The control bacterial proteins were an equivalent column fraction from a lysate of *E. coli* 294/pBR322 at twice the total protein concentration. The leukocyte interferon standard was Sendai virus induced interferon from normal human "buffy-coat" cells, purified chromatographically to a specific activity of 32 \times 10⁶ U/mg protein.

The control monkeys showed progressive lethargy, loss of balance, flaccid paralysis of the hind-limbs and watering of the eyes commencing around 8 hours prior to death. The interferon treated monkeys showed none of these abnormalities; they remained active at all times and developed no viremia (Table 3). The one monkey in the control group which did not develop viremia by 4 days died latest (164 h post-infection) but showed high titers of virus in the heart and brain on post-mortem. The interferon treated monkeys did not develop antibodies to EMC virus as determined 14 and 21 days after infection. These results demonstrate that the antiviral effects of Le-IF preparations in the infected animals can be attributed solely to interferon because the contaminating proteins are quite different in the bacterial and buffy coat preparations.

M. Isolation of cDNAs for Additional Leukocyte Interferons

DNA from the fully characterized Le-IF A cDNA-containing plasmid was excised with Pst I, isolated electrophoretically, and labelled by a published procedure (26) with ^{32}P . The resulting radioactively labelled DNA was used as a probe to screen-additional *E. coli* 294 transformants, obtained identically as those in Part D, by an in situ colony screening procedure (27). Colonies were isolated which hybridized in varying amounts to the probe. Plasmid DNA from these colonies and the ten hybridizing colonies referred to in Part I above was isolated by Pst cutting and characterized by three different methods. First, these Pst fragments were characterized by their restriction endonuclease digestion patterns with the enzymes Bgl II, Pvu II, and Eco RI. This analysis allowed the classification of at least eight different types (Le-IF A, Le-IF B, Le-IF C, Le-IF D, Le-IF E, Le-IF F, Le-IF G and Le-IF H), shown in FIG. 5, which approximates the location of various restriction cuts relative to the by-now known presequence and coding sequence of Le-IF A. One of these, Le-IF D, is believed to be identical to that reported in (39).

Secondly, certain of the DNAs were tested by a published hybridization selection assay (38) for the ability to selectively remove Le-IF mRNA from poly-A containing KG-1 cell RNA. Le-IF A, B, C and F were positive by this assay. Third, the latter Pst fragments were inserted in an expression plasmid, *E. coli* 294 transformed with the plasmid, and the fragments expressed. The expression products, believed to have been preinterferons, were all positive by CPE assay for interferon activity, albeit marginally active in the case of the Le-IF-F fragment. In addition to the foregoing, all of the Le-IF types described have been sequenced (See FIG. 3).

N. Direct Expression of a Second Mature Leukocyte Interferon

The sequence of the isolated fragment comprising the gene for mature Le-IF-B shows the first fourteen nucleotides of types A and B to be identical. We accordingly proposed to isolate a fragment from pLe-IF A25 bearing the trp-promoter-operator, ribosome binding site and the start of the Le-IF (A=B) gene, and combine this with the remaining portion of the B sequence in an expression plasmid. The salient restriction maps for the Pst fragment of pL4 (a plasmid comprising the Le-IF B Pst-ended gene depicted in FIG. 5) and pLe-IF A25 are shown, respectively, in FIGS. 7a and 7b.

To obtain the approximately 950 b.p. Sau 3a to Pst I fragment from the sequence shown in FIG. 7a several steps were necessary because of the presence of one or more intervening Sau 3a restriction sites, i.e.:

1. The following fragments were isolated:
 - a) 110b b.p. from Sau 3a to Eco RI;
 - b) 132 b.p. from Eco RI to Xba;
 - c) >700 b.p. from Xba to Pst.

2. Fragments (1a) and (1b) were ligated and cut with Xba and Bgl II to preclude self-polymerization through Sau 3a and Xba end terminals (the relevant Sau 3a site was within a Bgl II site; Bgl II cuts to leave a Sau 3a sticky end). A 242 b.p. fragment was isolated.

3. The product of (2) and (1c) were ligated and cut with Pst I and Bgl II, again to prevent self-polymerization. An approximate 950 b.p. fragment, Sau 3a to Pst I of FIG. 7a, was isolated. This fragment comprised that portion of the Le-IF B gene not common to Le-IF A.

4. An approximate 300 b.p. fragment (Hind III to Sau 3a) comprising the trp promoter-operator, ribosome binding site, ATG start signal and cysteine codon of Le-IF A was isolated from pLe-IF A25.

5. An approximately 3600 b.p. fragment Pst I to Hind III was isolated from pBr 322. This comprised the replicon and encoded tetracycline but not ampicillin resistance.

6. The fragments obtained in steps 3, 4 and 5 were triple-ligated and the resulting plasmid transformed into *E. coli* K-12 strain 294.

Transformants were miniscreened (37) and plasmid samples were digested with Eco RI. Digests yielded three fragments characteristic of:

1) The Eco RI-Eco RI trp promoter fragment; 2) The internal Eco RI to Eco RI fragment of pL4; and 3) protein translational start signal to Eco RI fragment of pL4.

In CPE assay, bacterial extracts from clones made in the foregoing fashion typically assay at about 10×10^6 units interferon activity per liter at $A_{550}=1$. One representative clone prepared in this manner is 294/pLIF B trp 7.

O. Direct Expression of Further Mature Leukocyte Interferons

Additional full-length gene fragments that comprise other Le-IF types may be tailored and placed in expression vehicles for expression as in the case of Le-IF A. Complete sequencing by conventional means will reveal whether a restriction site lies sufficiently near the first amino acid codon of the mature interferon type as to permit convenient resort to the approach employed in part J, supra, for the expression of mature Le-IF A, i.e., elimination of the presequence by restriction cutting and replacement of codons for the N-terminal amino acids lost in presequence elimination by ligation of a synthetic DNA fragment; Failing that, the procedure described in (36) may be employed. Briefly, this entails cleaving the presequence-containing fragment precisely before the point at which the codon for the first amino acid of the mature polypeptide begins, by:

1. converting the double stranded DNA to single-stranded DNA in a region surrounding that point;
2. hybridizing to the single-stranded region formed in step (a) a complementary primer length of single-stranded DNA, the 5' end of the primer lying opposite the nucleotide adjoining the intended cleavage site;
3. restoring that portion of the second strand eliminated in step 1 which lies in the 3' direction from the primer by reaction with DNA polymerase in the presence of adenine, thymine, guanine and cytosine-containing deoxynucleotide triphosphates; and
4. digesting the remaining single-stranded length of DNA which protrudes beyond the intended cleavage point.

A short length of synthetic DNA terminating, at the 3' end of the coding strand, with the translation start signal ATG can then be ligated by, e.g., blunt-end ligation to the resulting tailored gene for the mature interferons and the gene inserted into an expression plasmid and brought under the control of a promoter and its associated ribosome binding site.

Using the method described above, other probes can be used to advantage to isolate additional LeIF clones from the human genome. These, in turn, can be employed to produce additional leukocyte interferon proteins in accordance with this invention.

1. The 2000 base pair Eco RI fragment of the genomic clone (λ HLLeIF2) was subcloned into pBR325 at the Eco RI site. The resulting plasmid LeIF I was cleaved with Eco RI and the 2000 base pair fragment isolated. The deoxyoligonucleotide dAATTCTGCAG (SEQ ID NO:44) (an Eco RI>Pst I convertor) was ligated to the 2000 base pair Eco RI fragment and the resulting product cleaved with Pst I to give a 2000 base pair fragment containing Pst I ends. This was cleaved with Sau 96 and a 1100 base pair fragment isolated which has one Pst I end and one Sau 96 end.

2. The plasmid pLeIF C trp 35 was digested with Pst I and Xba I. The large fragment was isolated.

3. The small Xba I-Pst I fragment from pLeIF C trp 35 was digested with Xba I and Sau 96. A 40 base pair Xba I-Sau 96 fragment was isolated.

4. The fragments isolated in steps 1), 2) and 3) were ligated to form the expression plasmid pLeIF I trp 1.

LeIF-J

1. The plasmid pLeIF J contains a 3.8 kilobase Hind III fragment of human genomic DNA which includes the LeIF J gene sequence. A 760 base pair Dde I-Rsa I fragment was isolated from this plasmid.

2. The plasmid pLeIF B trp 7 was cleaved with Hind III and Dde I and a 340 bp Hind III-Dde I fragment isolated.

3. The plasmid pBR322 was cleaved with Pst I, blunt ended by incubation with DNA Pol I (Klenow fragment), then digested with Hind III. The large (~3600 bp) fragment was isolated.

4. Fragments isolated in steps 1), 2) and 3) were ligated to form the expression plasmid pLeIF J trp 1.

P. Purification

The content of leukocyte interferon in bacterial extracts may be enhanced by successive:

1. polyethylene-imine precipitation, in which most of the cellular protein, including the interferon, remains in the supernatant;
2. ammonium sulfate fractionation, in which interferon comes out of solution in 55 saturated ammonium sulfate;
3. suspension of the ammonium sulfate pellet in 0.06M potassium phosphate, 10 mM tris-HCl, pH 7.2, and dialysis against 25 mM tris-HCl, pH 7.9 (interferon activity remains in solution); and
4. Loading the above supernatant, pH adjusted to 8.5, on a DEAE-cellulose (Whatman DE-53) column and eluting with a linear gradient of 0 to 0.2M NaCl in 25 mM tris HCl, pH 8.5.
5. Adsorption on Cibachrome Blue Agarose (Amicon Blue A) or hydroxyapatite and elution with high salt (1.5 M KCl or 0.2 M phosphate respectively)—optional.
6. Molecular sizing on a Sephadex G-75 column.
7. Cation exchange chromatography on CM-cellulose (Whatman CM-52) in 25 mM ammonium acetate at pH 5.0, developed with an ammonium acetate gradient (to 0.2 M ammonium acetate).

In our hands, the above process gives essentially homogeneous material (e.g. >95 percent pure).

The material can also be further purified by further steps such as, in succession:

8. Size exclusion chromatography;
9. Reverse phase (RP-8) high pressure liquid chromatography; and if desired
10. Affinity chromatography on immobilized antiinterferon antibodies.

Affinity chromatography on a monoclonal antibody column can be used as an alternative to the Step 6 Sephadex G-75 column above. The material from step 4 is loaded on the monoclonal antibody column, prepared as described by Milstein, C., *Scientific American* 243, No. 4, p. 66 (1980), and eluted with 0.2 M acetic acid, 0.1 percent Triton and 0.15 M NaCl.

In an alternative, preferred embodiment, the leukocyte interferon produced by the procedures described herein can be purified by the following steps:

1. Frozen cell pellets containing the expressed leukocyte interferon are broken up manually or by appropriate size reduction equipment. The partially thawed cells are suspended in 4 volumes of buffer A. The suspension is held to approximately 4° C.

Buffer A:

- 0.1 M Tris adjusted to pH 7.5–8.0
- 10% (w/v) sucrose
- 0.2 M NaCl
- 5 mM EDTA
- 0.1 mM PMSF
- 10–100 mM MgCl₂

The suspension is passed through a Manton Gaulin laboratory homogenizer at about 6000 psi followed by a second pass at less than 1000 psi. Effluent from the homogenizer from both passes is cooled in an ice bath.

2. Polyethylene-imine (PEI) (e.g. Polymin P) is added slowly to the homogenate to a concentration of about 0.35% and allowed to stand for about 30 min. The solids are removed by centrifugation or filtration. This step is temperature controlled or performed sufficiently quickly that the supernatant (filtrate) is kept at less than 10° C. The supernatant (filtrate) is concentrated by ultrafiltration, e.g. on a Millipore Pellicon cassette system (PTGC, 5 ft.², MWCO 10,000), to approximately 1/10 the original volume. Particulate matter or haziness in the retentate may be removed by an appropriate filter such as a microporous membrane.

3. The clarified solution is loaded directly onto a monoclonal antibody column at a flux of 5–8 cm/hr. (e.g. 25–40 ml/hr. on 2.6 cm Diam column). After loading the column is washed with approximately 10 column volumes of 25 mM Tris HCl, pH 7.5–8.5 including NaCl (0.5M) and surfactant such as Triton X-100 (0.2%) or equivalent. Following the wash the column is rinsed with about 10 column volumes of solution containing 0.15 M NaCl and surfactant such as Triton X-100 (0.1%) or equivalent. The column is eluted with 0.2 M acetic acid containing surfactant such as Triton X-100 (0.1%) or equivalent. The protein peak from the monoclonal antibody column (as determined by UV absorbance or other convenient assay) is pooled and the pH adjusted to approximately 4.5 with 1 N NaOH or 1.0 M Tris base.

4. The pooled interferon peak is loaded onto a cationic exchanger such as Whatman CM52 cellulose or equivalent which has been equilibrated with suitable buffer such as ammonium acetate pH 4.5 (50 mM). After loading, the column is washed with equilibrating buffer until the UV absorbance of the effluent has reached a plateau so that little additional protein is eluting from the column. The column is then eluted with 25 mM ammonium acetate/0.12 M sodium

chloride or a combination which optimizes recovery of interferon and affords a lyophilized cake having satisfactory appearance and solubility properties.

The monoclonal antibodies employed in the preferred embodiment described above can be prepared by the procedures described by Staehelin, et. al., *P.N.A.S.*, 78, pp. 1848-52 (1981). Monoclonal antibodies are purified and covalently linked to Affigel-10 as described below:
Preparation and Purification of Monoclonal antibodies from Ascitic Fluid

Five female Balb/c mice were each inoculated with 5 to 10×10^6 hybridoma cells from mid-log growth phase. About 5×10^6 viable cells obtained from the mouse producing fluid were inoculated intraperitoneally into each of 10 or more mice. The ascitic fluid was collected repeatedly (2 to 4 times) from each mouse. Up to three transfers and collections may be performed from one group of mice to the next. Ascitic fluid from mice at each transfer was pooled.

Cells and debris were removed from the ascitic fluid by low speed centrifugation (500-1000xg) for 15 min. Then centrifugation was performed for 90 min. at 18,000 rpm in the SS34 Sorvall rotor without braking. The supernatant was frozen and stored at -20°C . After thawing, additional fibrin and particulate material were removed by centrifugation at 35,000 rpm for 90 min. in the Type 35 Spinco rotor. Batches of ascitic fluid from each transfer were tested for specific antibody activity by a solid phase antibody-binding assay (Staehelin, et. al., *P.N.A.S.*, 78, pp. 1848-52 (1981) and pooled if found satisfactory.

Concentration of protein in the pooled solutions was estimated by the approximation that 1 mg of protein yields an absorbance of 1.2 at 280 nm in a cuvette with a path length of 1.0 cm. Ascites fluids with high levels of antibody contain 30 to 35 mg protein/ml. This is equivalent to 4-7 mg of specific antibody/ml. The fluid was diluted with PBS (0.01 M sodium phosphate, pH 7.3, 0.15 M NaCl) to a protein concentration of 10 to 12 mg/ml (12 to 15 A_{280} units/ml).

To each 100 ml of diluted solution, 90 ml of room temperature saturated ammonium sulfate solution was added slowly with vigorous stirring at 0°C . The suspension was kept in ice for 40 to 60 min., then centrifuged for 15 min. at 10,000 rpm in a Sorvall GS-A rotor at 4°C . The supernatant was decanted and drained well. The protein pellets were dissolved in 0.02 M Tris.HCl (pH 7.9)/0.04 M NaCl (Buffer A; about 5 ml per 250 ml centrifuge bottle). The protein solution was dialyzed for 16 to 18 hrs. at room temperature against 100 volumes of Buffer A with at least one change of the buffer. The dialyzed solution was centrifuged at 15,000 rpm in a SS34 Sorvall rotor for 10 min. to remove undissolved material. About 30% to 35% of the original amount of total protein in the ascitic fluid was recovered as estimated by absorption at 280 nm.

The solution containing 30 to 40 mg of protein per ml was then applied to a column of DEAE-cellulose (DE52, Whatman) equilibrated with Buffer A. A column bed volume of at least 100 ml was used for each gram of protein applied. The antibody was eluted from the column with a linear NaCl gradient containing 0.02 M Tris.HCl, pH 7.9, from 0.04 M to 0.5 M NaCl. Pooled peak fractions eluting between 0.06 and 0.1 M NaCl were concentrated by precipitation with an equal volume of room temperature saturated ammonium sulfate and centrifugation. The protein pellets were dissolved in 0.2 M NaHCO_3 (pH ~ 8.0)/0.3 M NaCl (Buffer B) followed by dialysis against three changes of the same buffer at room temperature. The dialyzed solutions were centrifuged at 20,000xg for 15 min. to remove any insoluble

material. Protein concentration was adjusted to 20 to 25 mg/ml with Buffer B. SDS-polyacrylamide gel electrophoresis of representative monoclonal antibodies is shown in FIG. 1.

5 Preparation of Immunoabsorbants

Affigel-10 (BioRad Laboratories, Richmond, Calif.) was washed on a sintered glass filter three times with ice-cold isopropanol followed by three washes with ice-cold distilled water. The gel slurry ($\sim 50\%$ in cold water) was transferred to plastic tubes and sedimented by a brief centrifugation. The supernatant was aspirated. The packed gel was mixed with an equal volume of purified antibody solution and rotated end-over-end at 4°C . for 5 hrs. After reaction, the gel was centrifuged, then washed twice with Buffer C (0.1 M NaHCO_3 /0.15 M NaCl) to remove uncoupled antibody. Protein determination of the combined washes revealed that more than 90% of antibody was coupled to the gel.

To block unreacted sites, the gel was mixed with an equal volume of 0.1 M ethanolamine.HCl (pH 8) and rotated end-over-end at room temperature for 60 min. The gel slurry was washed free of reactants with PBS and stored in PBS in the presence of 0.02% (w/v) sodium azide at 4°C .

Q. Parenteral Administration

Le-IF may be parenterally administered to subjects requiring antitumor, or antiviral treatment, and to those exhibiting immunosuppressive conditions. Dosage and dose rats may parallel that currently in use in clinical investigations of human derived materials, e.g., about $(1-10) \times 10^6$ units daily, and in the case of materials of purity greater than 1 percent, likely up to, e.g., 50×10^6 units daily. Preliminary indications in the monkey study described above suggest that dosages of bacterially obtained Le-IF could be significantly elevated for greater effect owing to the essential absence of human proteins other than Le-IF, which proteins in leukocyte-derived materials may act as pyrogens, exhibiting adverse effects, e.g., malaise, temperature elevation, etc.

As one example of an appropriate dosage form for essentially homogeneous bacterial Le-IF in parenteral form, 3 mg. Le-IF of specific activity of, say, 2×10^8 U/mg may be dissolved in 25 ml. 5 N serum albumin (human)—USP, the solution passed through a bacteriological filter and the filtered solution aseptically subdivided into 100 vials, each containing 6×10^6 units pure interferon suitable for parenteral administration. The vials are preferably stored in the cold (-20°C .) prior to use.

The compounds of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the polypeptide hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are described in Remington's *Pharmaceutical Sciences* by E. W. Martin, which is hereby incorporated by reference. Such compositions will contain an effective amount of the interferon protein hereof together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions suitable for effective administration to the host. One preferred mode of administration is parenteral.

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			100					105					110			
Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val																
		115						120				125				
Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys																
	130					135					140					
Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro																

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145	150	155	160	
Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu				
	165	170	175	
Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu				
	180	185		
<210> SEQ ID NO 3				
<211> LENGTH: 1041				
<212> TYPE: DNA				
<213> ORGANISM: Homo sapiens				
<220> FEATURE:				
<221> NAME/KEY: CDS				
<222> LOCATION: (32)...(598)				
<400> SEQUENCE: 3				
tactagctca gcagcatggg caacatctac a atg gcc ttg act ttt tat tta				52
		Met Ala Leu Thr Phe Tyr Leu		
		1 5		
atg gtg gcc cta gtg gtg ctc agc tac aag tca ttc agc tct ctg ggc				100
Met Val Ala Leu Val Val Leu Ser Tyr Lys Ser Phe Ser Ser Leu Gly				
	10	15	20	
tgt gat ctg cct cag act cac agc ctg ggt aac agg agg gcc ttg ata				148
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile				
	25	30	35	
ctc ctg gca caa atg cga aga atc tct cct ttc tcc tgc ctg aag gac				196
Leu Leu Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp				
	40	45	50 55	
aga cat gac ttt gaa ttc ccc cag gag gag ttt gat gat aaa cag ttc				244
Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Asp Lys Gln Phe				
	60	65	70	
cag aag gct caa gcc atc tct gtc ctc cat gag atg atc cag cag acc				292
Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr				
	75	80	85	
ttc aac ctc ttc agc aca aag gac tca tct gct gct ttg gat gag acc				340
Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Leu Asp Glu Thr				
	90	95	100	
ctt cta gat gaa ttc tac atc gaa ctt gac cag cag ctg aat gac ctg				388
Leu Leu Asp Glu Phe Tyr Ile Glu Leu Asp Gln Gln Leu Asn Asp Leu				
	105	110	115	
gaa gtc ctg tgt gat cag gaa gtg ggg gtg ata gag tct ccc ctg atg				436
Glu Val Leu Cys Asp Gln Glu Val Gly Val Ile Glu Ser Pro Leu Met				
	120	125	130 135	
tac gag gac tcc atc ctg gct gtg agg aaa tac ttc caa aga atc act				484
Tyr Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr				
	140	145	150	
cta tat ctg aca gag aag aaa tac agc tct tgt gcc tgg gag gtt gtc				532
Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Ser Cys Ala Trp Glu Val Val				
	155	160	165	
aga gca gaa atc atg aga tcc ttc tct tta tca atc aac ttg caa aaa				580
Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ile Asn Leu Gln Lys				
	170	175	180	
aga ttg aag agt aag gaa tgagacctgg tacaacacgg aaatgattct				628
Arg Leu Lys Ser Lys Glu				
	185			
catagactaa tacagcagtc tacactttga caagttgtgc tctttcaaag acccttgttt				688
ctgccaaaac catgctatga attgaatcaa atgtgtcaag tgttttcagg agtgtaagc				748
aacatcctgt tcagctgtat gggcactagt cccttacaga tgaccatgct gatgatcta				808
ttcatctatt tatttaaadc tttatattagt taactactat aggacttaa attagttttg				868

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ttcatattat attatgtgaa cttttacatt gtgaattgtg taacaaaaac atgttcttat 928
atattattatt ttgccatggt tattaatatt ttactatgaa aaaattcttt atttattctt 988
taaaattgaa ctccaacca tgaattgtgc aaactgatta aagaatggat ggt 1041

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<210> SEQ ID NO 4
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 4

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Met Ala Leu Thr Phe Tyr Leu Met Val Ala Leu Val Val Leu Ser Tyr
 1           5           10          15
Lys Ser Phe Ser Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
          20           25           30
Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Arg Arg Ile Ser
          35           40           45
Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
 50           55           60
Glu Phe Asp Asp Lys Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65           70           75           80
His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser
          85           90           95
Ser Ala Ala Leu Asp Glu Thr Leu Leu Asp Glu Phe Tyr Ile Glu Leu
          100          105          110
Asp Gln Gln Leu Asn Asp Leu Glu Val Leu Cys Asp Gln Glu Val Gly
          115          120          125
Val Ile Glu Ser Pro Leu Met Tyr Glu Asp Ser Ile Leu Ala Val Arg
          130          135          140
Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
          145          150          155          160
Ser Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
          165          170          175
Leu Ser Ile Asn Leu Gln Lys Arg Leu Lys Ser Lys Glu
          180          185

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<210> SEQ ID NO 5
<211> LENGTH: 963
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (46)...(612)

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<400> SEQUENCE: 5

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aaggttatcc atctcaagta gcctagcaat atttgaaca tcca atg gcc ctg tcc 57
                               Met Ala Leu Ser
                               1
ttt tct tta ctt atg gcc gtg ctg gtg ctc agc tac aaa tcc atc tgt 105
Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile Cys
 5           10           15           20
tct ctg ggc tgt gat ctg cct cag acc cac agc ctc ggt aat agg agg 153
Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg
          25           30           35
gcc ttg ata ctc ctg gga caa atg gga aga atc tct cct ttc tcc tgc 201
Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys
          40           45           50
ctg aag gac aga cat gat ttc cga atc ccc cag gag gag ttt gat ggc 249
Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly

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55	60	65	
aac cag ttc cag aag gct caa gcc atc tct gtc ctc cat gag atg atc			297
Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile			
70	75	80	
cag cag acc ttc aat ctc ttc agc aca gag gac tca tct gct gct tgg			345
Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp			
85	90	95	100
gaa cag agc ctc cta gaa aaa ttt tcc act gaa ctt tac cag caa ctg			393
Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu			
105	110	115	
aat gac ctg gaa gca tgt gtg ata cag gag gtt ggg gtg gaa gag act			441
Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr			
120	125	130	
ccc ctg atg aat gag gac tcc atc ctg gct gtg agg aaa tac ttc caa			489
Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln			
135	140	145	
aga atc act ctt tat cta ata gag agg aaa tac agc cct tgt gcc tgg			537
Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp			
150	155	160	
gag gtt gtc aga gca gaa atc atg aga tcc ctc tcg ttt tca aca aac			585
Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn			
165	170	175	180
ttg caa aaa aga tta agg agg aag gat tgaaaactgg ttcaacatgg			632
Leu Gln Lys Arg Leu Arg Arg Lys Asp			
185			
caatgatcct gattgactaa tacattatct cacactttca cgagttcttc catttcaaag			692
actcacttct ataaccacaa acgcggtgaa tcaaaatddd caaatgtddd cagcagtgta			752
aagaagtgtc gtgtatacct gtgcaggcac tagtccttta cagatgacca ttctgatgtc			812
tctgttcacg ttttgtttaa atatttattt aattatddd aaaattdatg taatatcatg			872
agtcccttta cattgtggtt aatgtaacaa tatatgttct tcatattdag ccaatatatt			932
aatttccttd ttcattdaat ttttactata c			963

<210> SEQ ID NO 6

<211> LENGTH: 189

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1 5 10 15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
 20 25 30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser
 35 40 45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu
 50 55 60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
 65 70 75 80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
 85 90 95

Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
 100 105 110

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
 115 120 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg

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130	135	140	
Lys Tyr Phe Gln Arg	Ile Thr Leu Tyr Leu	Ile Glu Arg Lys Tyr Ser	
145	150	155	160
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser			
	165	170	175
Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp			
	180	185	
 <210> SEQ ID NO 7			
<211> LENGTH: 863			
<212> TYPE: DNA			
<213> ORGANISM: Homo sapiens			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (55)...(621)			
 <400> SEQUENCE: 7			
caaggttcag agtcacccat ctcagcaagc ccagaagtat ctgcaatatg tacg atg			57
		Met	
		1	
gcc tcg ccc ttt gct tta ctg atg gtc ctg gtg gtg ctc agc tgc aag			105
Ala Ser Pro Phe Ala Leu Leu Met Val Leu Val Val Leu Ser Cys Lys			
	5	10	15
tca agc tgc tct ctg ggc tgt gat ctg cct gag acc cac agc ctg gat			153
Ser Ser Cys Ser Leu Gly Cys Asp Leu Pro Glu Thr His Ser Leu Asp			
	20	25	30
aac agg agg acc ttg atg ctc ctg gca caa atg agc aga atc tct cct			201
Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser Pro			
	35	40	45
tcc tcc tgt ctg atg gac aga cat gac ttt gga ttt ccc cag gag gag			249
Ser Ser Cys Leu Met Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu			
	50	55	60
ttt gat ggc aac cag ttc cag aag gct cca gcc atc tct gtc ctc cat			297
Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro Ala Ile Ser Val Leu His			
	70	75	80
gag ctg atc cag cag atc ttc aac ctc ttt acc aca aaa gat tca tct			345
Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser			
	85	90	95
gct gct tgg gat gag gac ctc cta gac aaa ttc tgc acc gaa ctc tac			393
Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr			
	100	105	110
cag cag ctg aat gac ttg gaa gcc tgt gtg atg cag gag gag agg gtg			441
Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Met Gln Glu Glu Arg Val			
	115	120	125
gga gaa act ccc ctg atg aat gtg gac tcc atc ttg gct gtg aag aaa			489
Gly Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys Lys			
	130	135	140
tac ttc cga aga atc act ctc tat ctg aca gag aag aaa tac agc cct			537
Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro			
	150	155	160
tgt gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ctc tct tta			585
Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Leu			
	165	170	175
tca aca aac ttg caa gaa aga tta agg agg aag gaa taatatctgg			631
Ser Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu			
	180	185	
tccaacatga aaacaattct tattgactca tacaccaggt cagcgtttca tgaattctgt			691
catttcaaag actctcacc ctgctataac tatgaccatg ctgataaact gatttatcta			751
tttaaatatt tatttaacta ttcataagat ttaaattatt tttgttcata taacgtcatg			811

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tgcaccttta cactgtgggt agtgaataa aacatgttcc ttatatttac tc 863

<210> SEQ ID NO 8
 <211> LENGTH: 189
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Ala Ser Pro Phe Ala Leu Leu Met Val Leu Val Val Leu Ser Cys
 1 5 10 15
 Lys Ser Ser Cys Ser Leu Gly Cys Asp Leu Pro Glu Thr His Ser Leu
 20 25 30
 Asp Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser
 35 40 45
 Pro Ser Ser Cys Leu Met Asp Arg His Asp Phe Gly Phe Pro Gln Glu
 50 55 60
 Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro Ala Ile Ser Val Leu
 65 70 75 80
 His Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser
 85 90 95
 Ser Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu
 100 105 110
 Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Met Gln Glu Glu Arg
 115 120 125
 Val Gly Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys
 130 135 140
 Lys Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
 145 150 155 160
 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
 165 170 175
 Leu Ser Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu
 180 185

<210> SEQ ID NO 9
 <211> LENGTH: 876
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)...(510)

<400> SEQUENCE: 9

ctg cct ctg ggc tgt gat ctg cct cag gcc cac agc gtg ggt aac agg 48
 Leu Pro Leu Gly Cys Asp Leu Pro Gln Ala His Ser Val Gly Asn Arg
 1 5 10 15
 agg gcc ttc ata ctc ctg aca caa atg agg aga atc tct cct ttt tct 96
 Arg Ala Phe Ile Leu Leu Thr Gln Met Arg Arg Ile Ser Pro Phe Ser
 20 25 30
 tac ctg aag gac aga cat gac ttt gat ttt cca tca tca ggt gtt tca 144
 Tyr Leu Lys Asp Arg His Asp Phe Asp Phe Pro Ser Ser Gly Val Ser
 35 40 45
 tgg caa cca ctt cca gaa ggt tca agc tat ctt cct ttt cca tga gat 192
 Trp Gln Pro Leu Pro Glu Gly Ser Ser Tyr Leu Pro Phe Pro * Asp
 50 55 60
 gat gca gca gac ctt caa cct ctt cag cac aaa gga ctc atc tga tac 240
 Asp Ala Ala Asp Leu Gln Pro Leu Gln His Lys Gly Leu Ile * Tyr
 65 70 75
 ttg gga tga gac cct ttt aga caa atc cta cac tga act tta cca gca 288

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Leu Gly * Asp Pro Phe Arg Gln Ile Leu His * Thr Leu Pro Ala
80 85 90

gct gaa tga cct gga agc ctg tgt gat gta gaa ggt tgg agt gga aga 336
Ala Glu * Pro Gly Ser Leu Cys Asp Val Glu Gly Trp Ser Gly Arg
95 100 105

gac tcc cct gag gaa tgt gga ctc cat cct ggc tgt gag aaa ata ctt 384
Asp Ser Pro Glu Glu Cys Gly Leu His Pro Gly Cys Glu Lys Ile Leu
110 115 120

tca aag aat cac tct tta tct gac aaa gaa gaa gta tag ccc ttg ttc 432
Ser Lys Asn His Ser Leu Ser Asp Lys Glu Glu Val * Pro Leu Phe
125 130 135

ctg gga ggc tgt cag agc aga aat cat gag atc ctt ctc ttt atg aac 480
Leu Gly Gly Cys Gln Ser Arg Asn His Glu Ile Leu Leu Phe Met Asn
140 145 150

gaa ctt gca gga aag att aag gag gaa gga atgaaaactg gttcaacatg 530
Glu Leu Ala Gly Lys Ile Lys Glu Glu Gly
155 160

gaaatgagaa acatttccat gattaataca tcacttcaca cattcatgaa ttctgccatt 590

tgtcattttt gctatatcca tgacatgagt tgaatcaaaa ttttaaatg ttttcaggaa 650

tgtaagcag catcatgttc agctgtacag gcactagttc cttacggatg atcatgctga 710

tgatctgtt tatctatttg tctaaataat tatttaacta tttataatat ttaaaatctt 770

cttttcatgt atcatgtatt tttactttgt ggtaaatata acaacacatg ttctttatat 830

ttagtcaata tattactttg cttttttcat taaattttta ctatgg 876

<210> SEQ ID NO 10
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Leu Pro Leu Gly Cys Asp Leu Pro Gln Ala His Ser Val Gly Asn Arg
1 5 10 15

Arg Ala Phe Ile Leu Leu Thr Gln Met Arg Arg Ile Ser Pro Phe Ser
20 25 30

Tyr Leu Lys Asp Arg His Asp Phe Asp Phe Pro Ser Ser Gly Val Ser
35 40 45

Trp Gln Pro Leu Pro Glu Gly Ser Ser Tyr Leu Pro Phe Pro
50 55 60

<210> SEQ ID NO 11
<211> LENGTH: 985
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (9)...(575)

<400> SEQUENCE: 11

acatccca atg gcc ctg tcc ttt tct tta ctg atg gcc gtg ctg gtg ctc 50
Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu
1 5 10

agc tac aaa tcc atc tgt tct ctg ggc tgt gat ctg cct cag acc cac 98
Ser Tyr Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His
15 20 25 30

agc ctg ggt aat agg agg gcc ttg ata ctc ctg gca caa atg gga aga 146
Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg
35 40 45

atc tct cct ttc tcc tgc ctg aag gac aga cat gac ttt gga ttc ccc 194

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115	120	125	
Val Glu Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys			
130	135	140	
Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser			
145	150	155	160
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser			
	165	170	175
Leu Ser Lys Ile Phe Gln Glu Arg Leu Arg Arg Lys Glu			
	180	185	
<210> SEQ ID NO 13			
<211> LENGTH: 760			
<212> TYPE: DNA			
<213> ORGANISM: Homo sapiens			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)...(399)			
<400> SEQUENCE: 13			
cat gac ttt gga ttt cct cag gag gag ttt gat ggc aac cag ttc cag			48
His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln			
1	5	10	15
aag gct caa gcc atc tct gtc ctc cat gag atg atc cag cag acc ttc			96
Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr Phe			
	20	25	30
aat ctc ttc agc aca aag gac tca tct gct act tgg gat gag aca ctt			144
Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Asp Glu Thr Leu			
	35	40	45
cta gac aaa ttc tac act gaa ctt tac cag cag ctg aat gac ctg gaa			192
Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu			
	50	55	60
gcc tgt atg atg cag gag gtt gga gtg gaa gac act cct ctg atg aat			240
Ala Cys Met Met Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met Asn			
	65	70	75
gtg gac tct atc ctg act gtg aga aaa tac ttt caa aga atc acc ctc			288
Val Asp Ser Ile Leu Thr Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu			
	85	90	95
tat ctg aca gag aag aaa tac agc cct tgt gca tgg gag gtt gtc aga			336
Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg			
	100	105	110
gca gaa atc atg aga tcc ttc tct tta tca gca aac ttg caa gaa aga			384
Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn Leu Gln Glu Arg			
	115	120	125
tta agg agg aag gaa tgaaaactgg ttcaacatcg aaatgattct cattgactag			439
Leu Arg Arg Lys Glu			
	130		
tacaccattt cacacttctt gagttctgcc gtttcaaata ttaatttctg ctatatccat			499
gacttgagtt gaatcaaaat tttcaaactg tttcacacgt gttaagcaac acttctttag			559
ctgcacaggg actagtcttt tacagatgat catgctgaca tctattcttc tatttatcgt			619
catcattgtc gttttactac tattaatatt tatattatta ttgtttcatg ttatttttat			679
gtttagtttt agtttggtgt taatataaca aaatatgttt tgtggtcata tattaatttg			739
ctttttatta aattagtttg t			760

<210> SEQ ID NO 14
 <211> LENGTH: 133
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 14

His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln
 1 5 10 15
 Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr Phe
 20 25 30
 Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Asp Glu Thr Leu
 35 40 45
 Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
 50 55 60
 Ala Cys Met Met Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met Asn
 65 70 75 80
 Val Asp Ser Ile Leu Thr Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
 85 90 95
 Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
 100 105 110
 Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn Leu Gln Glu Arg
 115 120 125
 Leu Arg Arg Lys Glu
 130

<210> SEQ ID NO 15
 <211> LENGTH: 985
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (57)...(623)

<400> SEQUENCE: 15

ccaaggttca gtgttacc ccatcaacca gccagcagc atcttcggga ttcca atg 59
 Met
 1
 gca ttg ccc ttt gct tta atg atg gcc cta gtg gtg ctc agc tgc aag 107
 Ala Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys Lys
 5 10 15
 tca agc tgc tct ctg ggc tgt aat ctg tct caa acc cac agc ctg aat 155
 Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu Asn
 20 25 30
 aac agg agg act ttg atg ctc atg gca caa atg agg aga atc tct cct 203
 Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro
 35 40 45
 ttc tcc tgc ctg aag gac aga cat gac ttt gaa ttt ccc cag gag gaa 251
 Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu
 50 55 60 65
 ttt gat ggc aac cag ttc cag aaa gct caa gcc atc tct gtc ctc cat 299
 Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His
 70 75 80
 gag atg atg cag cag acc ttc aat ctc ttc agc aca aag aac tca tct 347
 Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser
 85 90 95
 gct gct tgg gat gag acc ctc cta gaa aaa ttc tac att gaa ctt ttc 395
 Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe
 100 105 110
 cag caa atg aat gac ctg gaa gcc tgt gtg ata cag gag gtt ggg gtg 443
 Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val
 115 120 125
 gaa gag act ccc ctg atg aat gag gac tcc atc ctg gct gtg aag aaa 491
 Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys Lys
 130 135 140 145

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tac ttc caa aga atc act ctt tat ctg atg gag aag aaa tac agc cct      539
Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro
                150                155                160

tgt gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ttc tct ttt      587
Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe
                165                170                175

tca aca aac ttg caa aaa aga tta agg agg aag gat tgaaaactgg          633
Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
                180                185

ttcatcatgg aatgattct cattgactaa tacatcatct cacactttca tgttcttcca    693

tttcaaagac tcacttctat aaccaccaca agttgaatca aaatttccaa atgttttcag    753

gagtgttaag aagcatcgtg tttacctgtg caggcactag tcctttacag atgaccattc    813

tgatgtctcc tttcatctat ttatttaaat atttatttat ttaactattt ttattattta    873

aattattttt tatgtaatat catgagtacc tttacattgt ggtaaatgta acaaatatgt    933

tcttcatatt tagccaatat attaatttcc tttttcatta aatttttact at          985

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<210> SEQ ID NO 16
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 16

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Met Ala Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys
 1                5                10                15

Lys Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu
 20                25                30

Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser
 35                40                45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
 50                55                60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
 65                70                75                80

His Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser
 85                90                95

Ser Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu
100                105                110

Phe Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
115                120                125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys
130                135                140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser
145                150                155                160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
165                170                175

Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
180                185

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<210> SEQ ID NO 17
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 17

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Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys
 1                5                10                15

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Lys Ser Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu
 20 25 30
 Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Lys Ile Ser
 35 40 45
 Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
 50 55 60
 Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
 65 70 75 80
 Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
 85 90 95
 Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
 100 105 110
 Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
 115 120 125
 Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys
 130 135 140
 Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
 145 150 155 160
 Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
 165 170 175
 Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
 180 185

<210> SEQ ID NO 18
 <211> LENGTH: 189
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Ala Leu Thr Phe Tyr Leu Met Val Ala Leu Val Val Leu Ser Tyr
 1 5 10 15
 Lys Ser Phe Ser Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
 20 25 30
 Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Arg Arg Ile Ser
 35 40 45
 Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
 50 55 60
 Glu Phe Asp Asp Lys Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
 65 70 75 80
 His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser
 85 90 95
 Ser Ala Ala Leu Asp Glu Thr Leu Leu Asp Glu Phe Tyr Ile Glu Leu
 100 105 110
 Asp Gln Gln Leu Asn Asp Leu Glu Val Leu Cys Asp Gln Glu Val Gly
 115 120 125
 Val Ile Glu Ser Pro Leu Met Tyr Glu Asp Ser Ile Leu Ala Val Arg
 130 135 140
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
 145 150 155 160
 Ser Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
 165 170 175
 Leu Ser Ile Asn Leu Gln Lys Arg Leu Lys Ser Lys Glu
 180 185

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<210> SEQ ID NO 19
 <211> LENGTH: 189
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1 5 10 15
 Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
 20 25 30
 Gly Asn Arg Arg Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser
 35 40 45
 Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu
 50 55 60
 Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
 65 70 75 80
 His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
 85 90 95
 Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
 100 105 110
 Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
 115 120 125
 Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
 130 135 140
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser
 145 150 155 160
 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
 165 170 175
 Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
 180 185

<210> SEQ ID NO 20
 <211> LENGTH: 189
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Ala Ser Pro Phe Ala Leu Leu Met Val Leu Val Val Leu Ser Cys
 1 5 10 15
 Lys Ser Ser Cys Ser Leu Gly Cys Asp Leu Pro Glu Thr His Ser Leu
 20 25 30
 Asp Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser
 35 40 45
 Pro Ser Ser Cys Leu Met Asp Arg His Asp Phe Gly Phe Pro Gln Glu
 50 55 60
 Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro Ala Ile Ser Val Leu
 65 70 75 80
 His Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser
 85 90 95
 Ser Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu
 100 105 110
 Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Met Gln Glu Glu Arg
 115 120 125
 Val Gly Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys
 130 135 140
 Lys Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser

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180 185

<210> SEQ ID NO 25
 <211> LENGTH: 1107
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (111)...(674)

<400> SEQUENCE: 25

gtatgttccc tatttaaggc taggcacaaa gcaaggtcct cagagaacct ggagcctaag 60

gttttaggctc acccatttca accagtctag cagcatctgc aacatctaca atg gcc 116
 Met Ala
 1

ttg acc ttt gct tta ctg gtg gcc ctc ctg gtg ctc agc tgc aag tca 164
 Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys Lys Ser
 5 10 15

agc tgc tct gtg ggc tgt gat ctg cct caa acc cac agc ctg ggt agc 212
 Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser
 20 25 30

agg agg acc ttg atg ctc ctg gca cag atg agg aga atc tct ctt ttc 260
 Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe
 35 40 45 50

tcc tgc ttg aag gac aga cat gac ttt gga ttt ccc cag gag gag ttt 308
 Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe
 55 60 65

ggc aac cag ttc caa aag gct gaa acc atc cct gtc ctc cat gag atg 356
 Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met
 70 75 80

atc cag cag atc ttc aat ctc ttc agc aca aag gac tca tct gct gct 404
 Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala
 85 90 95

tgg gat gag acc ctc cta gac aaa ttc tac act gaa ctc tac cag cag 452
 Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln
 100 105 110

ctg aat gac ctg gaa gcc tgt gtg ata cag ggg gtg ggg gtg aca gag 500
 Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu
 115 120 125 130

act ccc ctg atg aag gag gac tcc att ctg gct gtg agg aaa tac ttc 548
 Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe
 135 140 145

caa aga atc act ctc tat ctg aaa gag aag aaa tac agc cct tgt gcc 596
 Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
 150 155 160

tgg gag gtt gtc aga gca gaa atc atg aga tct ttt tct ttg tca aca 644
 Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr
 165 170 175

aac ttg caa gaa agt tta aga agt aag gaa tgaaaactgg ttcaacatgg 694
 Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
 180 185

aaatgatttt cattgattcg tatgccagct caccttttta tgatctgcca tttcaaagac 754

tcatgtttct gctatgacca tgacacgatt taaatctttt caaatgtttt taggagtatt 814

aatcaacatt gtattcagct ctttaaggcac tagtccctta cagaggacca tgctgactga 874

tccattatct atttaaatat ttttaaaata ttatttattt aactatttat aaaacaactt 934

atTTTTgttc atattatgtc atgtgcacct ttgcacagtg gttaatgtaa taaaatgtgt 994

tctttgtatt tggtaaattt attttTgtgtt gttcattgaa cttttgctat ggaacttttg 1054

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 tacttgttta ttctttaaaa tgaaattcca agcctaattg tgcaacctga tta 1107

<210> SEQ ID NO 26
 <211> LENGTH: 188
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys
 1 5 10 15
 Lys Ser Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu
 20 25 30
 Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser
 35 40 45
 Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
 50 55 60
 Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
 65 70 75 80
 Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
 85 90 95
 Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
 100 105 110
 Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
 115 120 125
 Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys
 130 135 140
 Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
 145 150 155 160
 Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
 165 170 175
 Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
 180 185

<210> SEQ ID NO 27
 <211> LENGTH: 1069
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (111)...(677)

<400> SEQUENCE: 27

gtatgttccc tatttaaggc taggcacaaa gcaaggtctt cagagaacct ggagcctaag 60
 gtttaggctc acccatttca accagtctag cagcatctgc aacatctaca atg gca 116
 Met Ala
 1
 ttg ccc ttt gct tta atg atg gcc ctg gtg gtg ctc agc tgc aag tca 164
 Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys Lys Ser
 5 10 15
 agc tgc tct ctg ggc tgt aat ctg tct caa acc cac agc ctg aat aac 212
 Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn
 20 25 30
 agg agg act ttg atg ctc atg gca caa atg agg aga atc tct cct ttc 260
 Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe
 35 40 45 50
 tcc tgc ctg aag gac aga cat gac ttt gaa ttt ccc cag gag gaa ttt 308
 Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe
 55 60 65

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gat ggc aac cag ttc cag aaa gct caa gcc atc tct gtc ctc cat gag	356
Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu	
70 75 80	
atg atg cag cag acc ttc aat ctc ttc agc aca aag aac tca tct gct	404
Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala	
85 90 95	
gct tgg gat gag acc ctc cta gaa aaa ttc tac att gaa ctt ttc cag	452
Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln	
100 105 110	
caa atg aat gac ctg gaa gcc tgt gtg ata cag gag gtt ggg gtg gaa	500
Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu	
115 120 125 130	
gag act ccc ctg atg aat gag gac tcc atc ctg gct gtg aag aaa tac	548
Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys Lys Tyr	
135 140 145	
ttc caa aga atc act ctt tat ctg atg gag aag aaa tac agc cct tgt	596
Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys	
150 155 160	
gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ctc tct ttt tca	644
Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser	
165 170 175	
aca aac ttg caa aaa aga tta agg agg aag gat tgaaaagtgg ttcatcatgg	697
Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp	
180 185	
aaatgattct cattgactaa tacatcatct cacactttca tgagttcttc catttcaaag	757
actcacttct cctataacca ccacaagttg aatcaaaatt ttcaaagtgt ttcaggagtg	817
taaagaagca tcatgtatac ctgtgcaggc actagtcctt tacagatgac catgctgatg	877
tctcctttca tctatttatt taaatattta tttatttaac tatttttatt atttaaatta	937
ttttttatgt taatatcatg tgtaccttta cattgtgggtt aatataacaa atatgttctt	997
catattttagc caatatatta atttcctttt tcattaaatt tttactatac aaaatttctg	1057
tgtttggtat tt	1069

<210> SEQ ID NO 28

<211> LENGTH: 189

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

Met Ala Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys	
1 5 10 15	
Lys Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu	
20 25 30	
Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser	
35 40 45	
Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu	
50 55 60	
Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu	
65 70 75 80	
His Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser	
85 90 95	
Ser Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu	
100 105 110	
Phe Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly	
115 120 125	
Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys	

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130	135	140	
Lys Tyr Phe Gln Arg	Ile Thr Leu Tyr Leu	Met Glu Lys Lys Tyr Ser	
145	150	155	160
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser			
	165	170	175
Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp			
	180	185	
<210> SEQ ID NO 29			
<211> LENGTH: 1112			
<212> TYPE: DNA			
<213> ORGANISM: Homo sapiens			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (113)...(679)			
<400> SEQUENCE: 29			
gtatgttcct tatttaagac ctatgcacag agcaaggtct tcagaaaacc tacaacc			60
ggttcagtgt taccctcat caaccagccc agcagcatct tcagggttcc ca atg gcc			118
		Met Ala	
		1	
ctg tcc ttt tct tta ctg atg gcc gtg ctg gtg ctc agc tac aaa tcc			166
Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser			
	5	10	15
atc tgt tct cta ggc tgt gat ctg cct cag acc cac agc ctg ggt aat			214
Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn			
	20	25	30
agg agg gcc ttg ata ctc ctg gca caa atg gga aga atc tct cct ttc			262
Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe			
	35	40	45
tcc tgc ctg aag gac aga cct gac ttt gga ctt ccc cag gag gag ttt			310
Ser Cys Leu Lys Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu Glu Phe			
	55	60	65
gat ggc aac cag ttc cag aag act caa gcc atc tct gtc ctc cat gag			358
Asp Gly Asn Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu			
	70	75	80
atg atc cag cag acc ttc aat ctc ttc agc aca gag gac tca tct gct			406
Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala			
	85	90	95
gct tgg gaa cag agc ctc cta gaa aaa ttt tcc act gaa ctt tac cag			454
Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln			
	100	105	110
caa ctg aat aac ctg gaa gca tgt gtg ata cag gag gtt ggg atg gaa			502
Gln Leu Asn Asn Leu Glu Ala Cys Val Ile Gln Glu Val Gly Met Glu			
	115	120	125
gag act ccc ctg atg aat gag gac tcc atc ctg gct gtg agg aaa tac			550
Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr			
	135	140	145
ttc caa aga atc act ctt tat cta aca gag aag aaa tac agc cct tca			598
Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Ser			
	150	155	160
gcc tgg gag gtt gtc aga gca gaa atc atg aga tct ctc tct ttt tca			646
Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser			
	165	170	175
aca aac ttg caa aaa ata tta agg agg aag gat tgaaaactgg ttcaacatgg			699
Thr Asn Leu Gln Lys Ile Leu Arg Arg Lys Asp			
	180	185	
caatgatcct gattgactaa tacattatct cacactttca tgagttcctc catttcaag			759
actcacttct ataaccacca cgagttgaat caaaattttc aaatgttttc agcagtgtaa			819

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agaagcgtcg tgtatacctg tgcaggcact agtactttac agatgacat gctgatgtct 879
ctgttcatct atttatttaa atatttattt aattattttt aagatttaaa ttattttttt 939
atgtaatatc atgtgtacct ttacattgtg gtgaatgtaa caatatatgt tcttcatatt 999
tagccaatat attaatttcc tttttcatta aatttttact atacaaaatt tcttgagttt 1059
gtttattctt aagaataaaa tgtcgaggct gactttacaa cctgacttaa aaa 1112

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<210> SEQ ID NO 30
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 30

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Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1           5           10          15
Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
 20          25          30
Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
 35          40          45
Pro Phe Ser Cys Leu Lys Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu
 50          55          60
Glu Phe Asp Gly Asn Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu
 65          70          75          80
His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
 85          90          95
Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
100         105         110
Tyr Gln Gln Leu Asn Asn Leu Glu Ala Cys Val Ile Gln Glu Val Gly
115         120         125
Met Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
130         135         140
Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
145         150         155         160
Pro Ser Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
165         170         175
Phe Ser Thr Asn Leu Gln Lys Ile Leu Arg Arg Lys Asp
180         185

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<210> SEQ ID NO 31
<211> LENGTH: 1109
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (111)...(677)

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<400> SEQUENCE: 31

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gtatgttcac tatttaaggc ctatgcacag agcaaagtct tcagaaaacc tagaggccaa 60
agttcaaggt taccatctc aagtagccta gcaacatttg caacatccca atg gcc 116
                               Met Ala
                               1
cgg tcc ttt tct tta ctg atg gtc gtg ctg gta ctc agc tac aaa tcc 164
Arg Ser Phe Ser Leu Leu Met Val Val Leu Val Leu Ser Tyr Lys Ser
 5           10           15
atc tgc tct ctg gcc tgt gat ctg cct cag acc cac agc ctg cgt aat 212
Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Arg Asn
 20          25          30

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agg agg gcc ttg ata ctc ctg gca caa atg gga aga atc tct cct ttc      260
Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe
 35                               40                               45                               50

tcc tgc ttg aag gac aga cat gaa ttc aga ttc cca gag gag gag ttt      308
Ser Cys Leu Lys Asp Arg His Glu Phe Arg Phe Pro Glu Glu Glu Phe
                    55                               60                               65

gat ggc cac cag ttc cag aag act caa gcc atc tct gtc ctc cat gag      356
Asp Gly His Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu
                    70                               75                               80

atg atc cag cag acc ttc aat ctc ttc agc aca gag gac tca tct gct      404
Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala
                    85                               90                               95

gct tgg gaa cag agc ctc cta gaa aaa ttt tcc act gaa ctt tac cag      452
Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln
 100                               105                               110

caa ctg aat gac ctg gaa gca tgt gtg ata cag gag gtt ggg gtg gaa      500
Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu
 115                               120                               125                               130

gag act ccc ctg atg aat gag gac ttc atc ctg gct gtg agg aaa tac      548
Glu Thr Pro Leu Met Asn Glu Asp Phe Ile Leu Ala Val Arg Lys Tyr
                    135                               140                               145

ttc caa aga atc act ctt tat cta atg gag aag aaa tac agc cct tgt      596
Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys
                    150                               155                               160

gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ttc tct ttt tca      644
Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser
 165                               170                               175

aca aac ttg aaa aaa gga tta agg agg aag gat tgaaaactgg ttcacatgg      697
Thr Asn Leu Lys Lys Gly Leu Arg Arg Lys Asp
 180                               185

aatgattct cattgactaa tgcacatct cacactttca tgagttcttc catttcaaag      757

actcacttct ataaccacca caagttgaat caaaatttcc aaatgttttc aggagtgtta      817

agaagcatcg tgtttacctg tgcaggcact agtcctttac agatgacat tctgatgtct      877

cctttcatct atttatttaa atatttattt atttaactat ttttattatt taaattatt      937

tttatgtaat atcatatgta cctttacatt gtggttaatg taacaaatat gttcttcata      997

tttagccaat atattaattt cctttttcat taaattttta ctatacaaaa tttcttgtgt      1057

ttgtttattt ttttaagatta aatgccaagc ctgactgtat aacctgactt aa      1109

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<210> SEQ ID NO 32

<211> LENGTH: 189

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

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Met Ala Arg Ser Phe Ser Leu Leu Met Val Val Leu Val Leu Ser Tyr
 1                               5                               10                               15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
 20                               25                               30

Arg Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
 35                               40                               45

Pro Phe Ser Cys Leu Lys Asp Arg His Glu Phe Arg Phe Pro Glu Glu
 50                               55                               60

Glu Phe Asp Gly His Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu
 65                               70                               75                               80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser

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165	170	175	
aac ttg caa aaa aga tta agg agg aag gat tgaaaactgg ttcaacatgg			696
Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp			
180	185		
caatgatcct gattgactaa tacattatct cacactttca tgagttcttc catttcaaag			756
actcacttct ataaccacga cgtggtgaat caaaattttc aaatgttttc agcagtgtaa			816
agaagtgtcg tgtatactg tgcaggcact agtcctttac agatgacat tctgatgtct			876
ctgttcatct tttgtttaa tatttattta attattttta aaatttatgt aatatcatga			936
gtcgctttac attgtggta atgtaacaat atatgttctt catatntagc caatatatta			996
atttcctttt tcattaaatt ttactatac aaaatttctt gtgtttgttt attctttaag			1056
ataaaatgcc aaggctgact ttacaacctg acttaaaaat agatgattta att			1109

<210> SEQ ID NO 34
 <211> LENGTH: 169
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

Ser	Leu	Gly	Cys	Asp	Leu	Pro	Gln	Thr	His	Thr	Leu	Arg	Asn	Arg	Arg
1			5					10						15	
Ala	Leu	Ile	Leu	Leu	Gly	Gln	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys
		20						25					30		
Leu	Lys	Asp	Arg	His	Asp	Phe	Arg	Ile	Pro	Gln	Glu	Glu	Phe	Asp	Gly
	35					40					45				
Asn	Gln	Phe	Gln	Lys	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met	Ile
	50				55						60				
Gln	Gln	Thr	Phe	Asn	Leu	Phe	Ser	Thr	Glu	Asp	Ser	Ser	Ala	Ala	Trp
65				70					75						80
Glu	Gln	Ser	Leu	Leu	Glu	Lys	Phe	Ser	Thr	Glu	Ile	Tyr	Gln	Gln	Leu
			85					90					95		
Asn	Asp	Leu	Glu	Ala	Cys	Val	Ile	Gln	Glu	Val	Gly	Val	Glu	Glu	Thr
		100						105					110		
Pro	Leu	Met	Asn	Glu	Asp	Ser	Ile	Leu	Ala	Val	Arg	Lys	Tyr	Phe	Gln
		115					120					125			
Arg	Ile	Thr	Leu	Tyr	Leu	Ile	Glu	Arg	Lys	Tyr	Ser	Pro	Cys	Ala	Trp
	130					135					140				
Glu	Val	Val	Arg	Ala	Glu	Ile	Met	Arg	Ser	Leu	Ser	Phe	Ser	Thr	Asn
145					150					155					160
Leu	Gln	Lys	Arg	Leu	Arg	Arg	Lys	Asp							
				165											

<210> SEQ ID NO 35
 <211> LENGTH: 188
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Met	Ala	Leu	Thr	Phe	Ala	Leu	Leu	Val	Ala	Leu	Leu	Val	Leu	Ser	Cys
1				5					10					15	
Lys	Ser	Ser	Cys	Ser	Val	Gly	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu
			20					25					30		
Gly	Ser	Arg	Arg	Thr	Leu	Met	Leu	Leu	Ala	Gln	Met	Arg	Arg	Ile	Ser
		35				40						45			
Leu	Phe	Ser	Cys	Leu	Lys	Asp	Arg	His	Asp	Phe	Gly	Phe	Pro	Gln	Glu

-continued

50	55	60															
Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His																	
65	70	75															80
Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser																	
	85	90															95
Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr																	
	100	105															110
Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val																	
	115	120															125
Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys																	
	130	135															140
Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro																	
	145	150															155
Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu																	
	165	170															175
Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu																	
	180	185															

<210> SEQ ID NO 36
 <211> LENGTH: 189
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Met Ala Leu Pro Phe Ser Leu Met Met Ala Leu Val Val Leu Ser Cys																	
1	5	10															15
Lys Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu																	
	20	25															30
Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser																	
	35	40															45
Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu																	
	50	55															60
Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu																	
	65	70															80
His Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser																	
	85	90															95
Ser Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu																	
	100	105															110
Phe Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly																	
	115	120															125
Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg																	
	130	135															140
Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser																	
	145	150															155
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser																	
	165	170															175
Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp																	
	180	185															

<210> SEQ ID NO 37
 <211> LENGTH: 189
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr

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1	5	10	15
Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu	20	25	30
Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser	35	40	45
Pro Phe Ser Cys Leu Lys Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu	50	55	60
Glu Phe Asp Gly Asn Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu	65	70	75
His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser	85	90	95
Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu	100	105	110
Tyr Gln Gln Leu Asn Asn Leu Glu Ala Cys Val Ile Gln Glu Val Gly	115	120	125
Met Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg	130	135	140
Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser	145	150	155
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser	165	170	175
Phe Ser Thr Asn Leu Gln Lys Ile Leu Arg Arg Lys Asp	180	185	

<210> SEQ ID NO 38
 <211> LENGTH: 169
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg	1	5	10	15
Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys	20	25	30	
Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly	35	40	45	
Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile	50	55	60	
Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp	65	70	75	80
Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln Leu	85	90	95	
Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr	100	105	110	
Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln	115	120	125	
Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp	130	135	140	
Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn	145	150	155	160
Leu Gln Lys Arg Leu Arg Arg Lys Asp	165			

<210> SEQ ID NO 39
 <211> LENGTH: 12

-continued

<212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Eco RI linker

 <400> SEQUENCE: 39

 catgaattca tg 12

 <210> SEQ ID NO 40
 <211> LENGTH: 14
 <212> TYPE: DNA
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: XbaI and EcoRI cleavage sites

 <400> SEQUENCE: 40

 tctagaattc tatg 14

 <210> SEQ ID NO 41
 <211> LENGTH: 14
 <212> TYPE: DNA
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: XbaI and EcoRI cleavage sites

 <400> SEQUENCE: 41

 catagaattc taga 14

 <210> SEQ ID NO 42
 <211> LENGTH: 11
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic deoxyoligonucleotides

 <400> SEQUENCE: 42

 aattcatgtg t 11

 <210> SEQ ID NO 43
 <211> LENGTH: 11
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic deoxyoligonucleotides

 <400> SEQUENCE: 43

 gatcacacat g 11

 <210> SEQ ID NO 44
 <211> LENGTH: 10
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EcoRI to PstI convertor

 <400> SEQUENCE: 44

 aattctgcag 10

 <210> SEQ ID NO 45
 <211> LENGTH: 166
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

-continued

<400> SEQUENCE: 45

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
 1 5 10 15
 Met Leu Leu Ala Gln Met Arg Lys Ile Ser Leu Phe Ser Cys Leu Lys
 20 25 30
 Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
 35 40 45
 Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
 50 55 60
 Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65 70 75 80
 Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
 100 105 110
 Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
 115 120 125
 Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
 130 135 140
 Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 145 150 155 160
 Ser Leu Arg Ser Lys Glu
 165

<210> SEQ ID NO 46

<211> LENGTH: 167

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: SITE

<222> LOCATION: (1)...(1)

<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 46

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1 5 10 15
 Ile Leu Leu Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
 20 25 30
 Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Asp Lys Gln
 35 40 45
 Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
 50 55 60
 Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Leu Asp Glu
 65 70 75 80
 Thr Leu Leu Asp Glu Phe Tyr Ile Glu Leu Asp Gln Gln Leu Asn Asp
 85 90 95
 Leu Glu Val Leu Cys Asp Gln Glu Val Gly Val Ile Glu Ser Pro Leu
 100 105 110
 Met Tyr Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
 115 120 125
 Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Ser Cys Ala Trp Glu Val
 130 135 140
 Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ile Asn Leu Gln
 145 150 155 160
 Lys Arg Leu Lys Ser Lys Glu
 165

-continued

<210> SEQ ID NO 47
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

 <400> SEQUENCE: 47

 Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1 5 10 15
 Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
 20 25 30
 Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly Asn Gln
 35 40 45
 Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
 50 55 60
 Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
 65 70 75 80
 Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp
 85 90 95
 Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
 100 105 110
 Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
 115 120 125
 Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp Glu Val
 130 135 140
 Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
 145 150 155 160
 Lys Arg Leu Arg Arg Lys Asp
 165

<210> SEQ ID NO 48
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

 <400> SEQUENCE: 48

 Xaa Cys Asp Leu Pro Glu Thr His Ser Leu Asp Asn Arg Arg Thr Leu
 1 5 10 15
 Met Leu Leu Ala Gln Met Ser Arg Ile Ser Pro Ser Ser Cys Leu Met
 20 25 30
 Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
 35 40 45
 Phe Gln Lys Ala Pro Ala Ile Ser Val Leu His Glu Leu Ile Gln Gln
 50 55 60
 Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu
 65 70 75 80
 Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp
 85 90 95
 Leu Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu
 100 105 110

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Met Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile
 115 120 125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
 130 135 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln
 145 150 155 160

Glu Arg Leu Arg Arg Lys Glu
 165

<210> SEQ ID NO 49
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 49

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1 5 10 15

Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
 20 25 30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
 35 40 45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
 50 55 60

Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Glu Gln
 65 70 75 80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Asn Gln Gln Leu Asn Asp
 85 90 95

Met Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
 100 105 110

Met Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile
 115 120 125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
 130 135 140

Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Lys Ile Phe Gln
 145 150 155 160

Glu Arg Leu Arg Arg Lys Glu
 165

<210> SEQ ID NO 50
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 50

Xaa Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu
 1 5 10 15

Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
 20 25 30

Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
 35 40 45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln

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50	55	60																		
Thr	Phe	Asn	Leu	Phe	Ser	Thr	Lys	Asn	Ser	Ser	Ala	Ala	Trp	Asp	Glu					
65					70					75				80						
Thr	Leu	Leu	Glu	Lys	Phe	Tyr	Ile	Glu	Leu	Phe	Gln	Gln	Met	Asn	Asp					
				85					90					95						
Leu	Glu	Ala	Cys	Val	Ile	Gln	Glu	Val	Gly	Val	Glu	Glu	Thr	Pro	Leu					
			100					105					110							
Met	Asn	Glu	Asp	Ser	Ile	Leu	Ala	Val	Arg	Lys	Tyr	Phe	Gln	Arg	Ile					
		115					120					125								
Thr	Leu	Tyr	Leu	Met	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala	Trp	Glu	Val					
	130					135					140									
Val	Arg	Ala	Glu	Ile	Met	Arg	Ser	Phe	Ser	Phe	Ser	Thr	Asn	Leu	Gln					
145					150					155					160					
Lys	Arg	Leu	Arg	Arg	Lys	Asp														
				165																

<210> SEQ ID NO 51
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 51

Xaa	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu	Gly	Asn	Arg	Arg	Ala	Leu					
1				5					10					15						
Ile	Leu	Leu	Ala	Gln	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu	Lys					
			20				25						30							
Asp	Arg	Pro	Asp	Phe	Gly	Leu	Pro	Gln	Glu	Glu	Phe	Asp	Gly	Asn	Gln					
		35					40					45								
Phe	Gln	Lys	Thr	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met	Ile	Gln	Gln					
	50					55					60									
Thr	Phe	Asn	Leu	Phe	Ser	Thr	Glu	Asp	Ser	Ser	Ala	Ala	Trp	Glu	Gln					
65					70					75				80						
Ser	Leu	Leu	Glu	Lys	Phe	Ser	Thr	Glu	Leu	Tyr	Gln	Gln	Leu	Asn	Asn					
				85					90					95						
Leu	Glu	Ala	Cys	Val	Ile	Gln	Glu	Val	Gly	Met	Glu	Glu	Thr	Pro	Leu					
			100					105					110							
Met	Asn	Glu	Asp	Ser	Ile	Leu	Ala	Val	Arg	Lys	Tyr	Phe	Gln	Arg	Ile					
		115					120					125								
Thr	Leu	Tyr	Leu	Thr	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala	Trp	Glu	Val					
	130					135					140									
Val	Arg	Ala	Glu	Ile	Met	Arg	Ser	Leu	Ser	Phe	Ser	Thr	Asn	Leu	Gln					
145					150					155					160					
Lys	Ile	Leu	Arg	Arg	Lys	Asp														
				165																

<210> SEQ ID NO 52
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 52

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Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Arg Asn Arg Arg Ala Leu
 1           5           10           15
Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
 20           25           30
Asp Arg His Glu Phe Arg Phe Pro Glu Glu Glu Phe Asp Gly His Gln
 35           40           45
Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
 50           55           60
Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
 65           70           75           80
Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp
 85           90           95
Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
 100          105          110
Met Asn Glu Asp Phe Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
 115          120          125
Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
 130          135          140
Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr Asn Leu Lys
 145          150          155          160
Lys Gly Leu Arg Arg Lys Asp
 165

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<210> SEQ ID NO 53
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

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<400> SEQUENCE: 53

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Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
 1           5           10           15
Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
 20           25           30
Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
 35           40           45
Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
 50           55           60
Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65           70           75           80
Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
 85           90           95
Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
 100          105          110
Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
 115          120          125
Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
 130          135          140
Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 145          150          155          160
Ser Leu Arg Ser Lys Glu
 165

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<210> SEQ ID NO 54
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 54

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1             5             10             15
Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
      20             25             30
Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly Asn Gln
      35             40             45
Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
      50             55             60
Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
      65             70             75             80
Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln Leu Asn Asp
      85             90             95
Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
      100            105            110
Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
      115            120            125
Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp Glu Val
      130            135            140
Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
      145            150            155            160
Lys Arg Leu Arg Arg Lys Asp
      165

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<210> SEQ ID NO 55
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 55

Xaa Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu
 1             5             10             15
Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
      20             25             30
Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
      35             40             45
Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln
      50             55             60
Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala Ala Trp Asp Glu
      65             70             75             80
Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln Gln Met Asn Asp
      85             90             95
Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
      100            105            110
Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile

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115	120	125																		
Thr	Leu	Tyr	Leu	Met	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala	Trp	Glu	Val					
	130					135					140									
Val	Arg	Ala	Glu	Ile	Met	Arg	Ser	Leu	Ser	Phe	Ser	Thr	Asn	Leu	Gln					
145					150					155					160					
Lys	Arg	Leu	Arg	Arg	Lys	Asp														
				165																

<210> SEQ ID NO 56
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 56

Xaa	Cys	Asn	Leu	Ser	Gln	Thr	His	Ser	Leu	Asn	Asn	Arg	Arg	Thr	Leu					
1				5					10					15						
Met	Leu	Met	Ala	Gln	Met	Arg	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu	Lys					
			20					25					30							
Asp	Arg	His	Asp	Phe	Glu	Phe	Pro	Gln	Glu	Glu	Phe	Asp	Gly	Asn	Gln					
		35					40					45								
Phe	Gln	Lys	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met	Met	Gln	Gln					
	50					55					60									
Thr	Phe	Asn	Leu	Phe	Ser	Thr	Lys	Asn	Ser	Ser	Ala	Ala	Trp	Asp	Glu					
65				70						75					80					
Thr	Leu	Leu	Glu	Lys	Phe	Tyr	Ile	Glu	Leu	Phe	Gln	Gln	Met	Asn	Asp					
				85					90					95						
Leu	Glu	Ala	Cys	Val	Ile	Gln	Glu	Val	Gly	Val	Glu	Glu	Thr	Pro	Leu					
		100						105					110							
Met	Asn	Glu	Asp	Ser	Ile	Leu	Ala	Val	Lys	Lys	Tyr	Phe	Gln	Arg	Ile					
		115					120					125								
Thr	Leu	Tyr	Leu	Met	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala	Trp	Glu	Val					
	130					135					140									
Val	Arg	Ala	Glu	Ile	Met	Arg	Ser	Phe	Ser	Phe	Ser	Thr	Asn	Leu	Gln					
145					150					155					160					
Lys	Arg	Leu	Arg	Arg	Lys	Asp														
				165																

<210> SEQ ID NO 57
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 57

Xaa	Cys	Asp	Leu	Pro	Gln	Thr	His	Thr	Leu	Arg	Asn	Arg	Arg	Ala	Leu					
1				5					10					15						
Ile	Leu	Leu	Gly	Gln	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu	Lys					
			20					25					30							
Asp	Arg	His	Asp	Phe	Arg	Ile	Pro	Gln	Glu	Glu	Phe	Asp	Gly	Asn	Gln					
		35				40						45								
Phe	Gln	Lys	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met	Ile	Gln	Gln					
	50					55					60									

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Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
 65 70 75 80
 Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln Leu Asn Asp
 85 90 95
 Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
 100 105 110
 Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
 115 120 125
 Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp Glu Val
 130 135 140
 Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
 145 150 155 160
 Lys Arg Leu Arg Arg Lys Asp
 165

<210> SEQ ID NO 58
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 58

Xaa Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu
 1 5 10 15
 Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
 20 25 30
 Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
 35 40 45
 Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln
 50 55 60
 Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala Ala Trp Asp Glu
 65 70 75 80
 Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln Gln Met Asn Asp
 85 90 95
 Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
 100 105 110
 Met Asn Glu Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile
 115 120 125
 Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
 130 135 140
 Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
 145 150 155 160
 Lys Arg Leu Arg Arg Lys Asp
 165

<210> SEQ ID NO 59
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (1)...(1)
 <223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 59

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Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1 5 10 15
 Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
 20 25 30
 Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu Glu Phe Asp Gly Asn Gln
 35 40 45
 Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
 50 55 60
 Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
 65 70 75 80
 Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asn
 85 90 95
 Leu Glu Ala Cys Val Ile Gln Glu Val Gly Met Glu Glu Thr Pro Leu
 100 105 110
 Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
 115 120 125
 Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Ser Ala Trp Glu Val
 130 135 140
 Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
 145 150 155 160
 Lys Ile Leu Arg Arg Lys Asp
 165

<210> SEQ ID NO 60
 <211> LENGTH:
 <212> TYPE:
 <213> ORGANISM:

<400> SEQUENCE: 60

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<210> SEQ ID NO 61
 <211> LENGTH:
 <212> TYPE:
 <213> ORGANISM:

<400> SEQUENCE: 61

000

<210> SEQ ID NO 62
 <211> LENGTH: 15
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

Asp Asp Ala Ala Asp Leu Gln Pro Leu Gln His Lys Gly Leu Ile
 1 5 10 15

<210> SEQ ID NO 63
 <211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Asp Pro Phe Arg Gln Ile Leu His
 1 5

<210> SEQ ID NO 64
 <211> LENGTH: 6
 <212> TYPE: PRT

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Thr Leu Pro Ala Ala Glu
1 5

<210> SEQ ID NO 65

<211> LENGTH: 41

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Pro Gly Ser Leu Cys Asp Val Glu Gly Trp Ser Gly Arg Asp Ser Pro
1 5 10 15

Glu Glu Cys Gly Leu His Pro Gly Cys Glu Lys Ile Leu Ser Lys Asn
20 25 30

His Ser Leu Ser Asp Lys Glu Glu Val
35 40

<210> SEQ ID NO 66

<211> LENGTH: 29

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 66

Pro Leu Phe Leu Gly Gly Cys Gln Ser Arg Asn His Glu Ile Leu Leu
1 5 10 15

Phe Met Asn Glu Leu Ala Gly Lys Ile Lys Glu Glu Gly
20 25

<210> SEQ ID NO 67

<211> LENGTH: 52

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Lys Val Gly Val Glu Glu Thr Pro Leu Arg Asn Val Asp Ser Ile Leu
1 5 10 15

Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Lys Lys
20 25 30

Lys Tyr Ser Pro Cys Ser Trp Glu Ala Val Arg Ala Glu Ile Met Arg
35 40 45

Ser Phe Ser Leu
50

<210> SEQ ID NO 68

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu
1 5 10

<210> SEQ ID NO 69

<211> LENGTH: 19

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
1 5 10 15

-continued

Lys Ser Ile

<210> SEQ ID NO 70
 <211> LENGTH: 19
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1 5 10 15

Lys Ser Ile

What is claimed is:

1. A DNA comprising a nucleotide sequence encoding a polypeptide of 165–166 amino acids having the amino acid sequence of a mature human leukocyte interferon unaccompanied by any corresponding presequence or portion thereof, wherein said nucleotide sequence further comprises an ATG immediately preceding the codon corresponding to the amino-terminal amino acid of said polypeptide.

2. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:45, wherein the amino-terminal amino acid is cysteine.

3. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:46, wherein the amino-terminal amino acid is cysteine.

4. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:47, wherein the amino-terminal amino acid is cysteine.

5. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:49, wherein the amino-terminal amino acid is cysteine.

6. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:50, wherein the amino-terminal amino acid is cysteine.

7. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:51, wherein the amino-terminal amino acid is cysteine.

8. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:52, wherein the amino-terminal amino acid is cysteine.

9. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:48, wherein the amino-terminal amino acid is cysteine.

10. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:53, wherein the amino-terminal amino acid is cysteine.

11. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:54, wherein the amino-terminal amino acid is cysteine.

12. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:55, wherein the amino-terminal amino acid is cysteine.

13. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:56, wherein the amino-terminal amino acid is cysteine.

14. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:57, wherein the amino-terminal amino acid is cysteine.

15. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:58, wherein the amino-terminal amino acid is cysteine.

16. The DNA according to claim 1, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:59, wherein the amino-terminal amino acid is cysteine.

17. A process for producing a polypeptide product, comprising culturing a bacterium transformed with a replicable bacterial expression vehicle comprising a DNA according to any of claims 1–16, said vehicle capable of expressing in said bacterium a mature human leukocyte interferon having about 165–166 amino acids unaccompanied by any corresponding presequence or portion thereof.

18. A process for producing a polypeptide product, comprising culturing a bacterium transformed with a replicable bacterial expression vehicle comprising a DNA according to claim 2, said vehicle capable of expressing in said bacterium a mature human leukocyte interferon having about 165–166 amino acids unaccompanied by any corresponding presequence or portion thereof.

19. A process for producing a polypeptide product, comprising culturing a bacterium transformed with a replicable bacterial expression vehicle comprising a DNA according to claim 10, said vehicle capable of expressing in said bacterium a mature human leukocyte interferon having about 165–166 amino acids unaccompanied by any corresponding presequence or portion thereof.

20. A bacterium transformed with a DNA according to claims 1–16.

21. A bacterium according to claim 20, wherein the bacterium is *E. coli*.

22. A bacterium according to claim 20, wherein the bacterium is *E. coli* K-12 strain 294.

* * * * *